Research Square

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Urinary cathepsin A as a non-invasive diagnostic biomarker in heart failure

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Abstract

Background

Heart failure (HF) still lacks a simple, repeatable test that can be applied outside specialist centres. Urine collects protein changes linked to disease, but whether urinary lysosomal cathepsin A (CTSA) can help diagnose HF is unknown. We assessed urinary CTSA as a potential diagnostic marker for HF.

Methods

We enrolled 58 HF patients and 50 age- and sex-matched healthy controls. Urine from 10 patients and 10 controls was analyzed by 4D label-free quantitative (4D-LFQ) proteomics; the remaining samples were used for ELISA confirmation. Differentially expressed proteins were identified and subjected to Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein–protein interaction (PPI) analyses. CTSA abundance was measured by ELISA and evaluated by regression and receiver operating characteristic (ROC) analysis, with adjustment for renal function.

Results

4D-LFQ identified 200 differentially expressed proteins (82 up- and 118 downregulated; fold change > 1.5, $P < 0.05$). Bioinformatic analysis highlighted CTSA, a lysosomal protease in the renin–angiotensin system. ELISA showed that urinary CTSA was higher in HF than in controls (median [IQR] 3055.43 [2498.33–3434.76] vs 2551.4 [2084.76–2785.9] pg/mL; $P < 0.0001$). The CTSA/eGFR ratio discriminated HF from controls (AUC 0.89, 95% CI 0.83–0.96; sensitivity 85.0%, specificity 81.25%). The association remained positive after adjustment for eGFR ($\beta = 524.098$, $P = 0.001$). PPI analysis placed CTSA at the centre of a cluster of co-expressed lysosomal hydrolases and peptidases.

Conclusions

Urinary CTSA is increased in HF and, when interpreted with renal function, performs as a non-invasive diagnostic marker. It complements blood-based assays and merits validation in larger cohorts.

1 Introduction

Heart failure (HF) is a clinical syndrome that follows coronary artery disease, hypertension, and cardiomyopathy, and drives myocardial hypertrophy, fibrosis, and remodelling [1]. In China, about 13.7 million adults over 35 years live with HF, and the number grows as the population ages and risk factors such as hypertension and diabetes rise [2]. HF often starts silently and is diagnosed late because its symptoms are non-specific, which worsens outcomes [3]. Current tools—cardiac imaging and blood biomarkers such as BNP and NT-proBNP—are useful but leave gaps in early and repeatable detection [4].

Urine is a useful alternative. It is produced by glomerular filtration and can be collected repeatedly without invasion; its protein content stays fairly stable over time [5]. Urinary peptides have been linked to left ventricular diastolic dysfunction [6], so the urine proteome can mirror cardiac change. One problem is renal function. HF often changes glomerular filtration and tubular handling, so urine concentrations may reflect the kidney more than the heart. Any urinary marker therefore needs to be interpreted in the context of renal function.

We used 4D label-free quantitative (4D-LFQ) proteomics to compare the urinary protein profiles of HF patients and healthy controls and to find candidate markers. Among the proteins that changed, lysosomal cathepsin A (CTSA) stood out. CTSA is a lysosomal protease with several functions and has been linked to cardiovascular disease. We measured urinary CTSA by ELISA and tested its diagnostic value, paying close attention to confounding by renal function.

2 Methods

2.1 Collection and preparation of urine samples

We enrolled 58 patients with HF, all aged ≥ 18 years, with a clinically measured left ventricular ejection fraction (LVEF) < 45% and plasma NT-proBNP ≥ 900 pg/mL. Cardiac function was classified by the New York Heart Association (NYHA) into classes II, III, or IV. We also enrolled 50 age- and sex-matched healthy controls with LVEF ≥ 50% and no organic heart disease. The study was approved by the Institutional Review Board of [Medical Research Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China] (protocol AF/SC-14/05.0, April 9,2025) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Urine (10 mL) was collected in the morning, placed in sterile tubes, and centrifuged at 2500 × g for 10 min at 4°C. The supernatants were stored at − 80°C after removing debris and particulates. For 4D-LFQ we used samples from 10 HF patients and 10 healthy controls chosen at random; the rest were used for ELISA confirmation.

2.2 Proteomic analysis

Urine proteins were concentrated to 2 mL by ultrafiltration. We added an equal volume of methanol and one-quarter volume of chloroform, then centrifuged at 12,000 × g for 15 min at room temperature. The pellet was washed with methanol and redissolved in 50 µL of 1% sodium deoxycholate. Protein concentration was measured with a Beyotime (China) kit. Proteins were reduced with 5 mM dithiothreitol at 37°C for 1 h and alkylated with 15 mM iodoacetamide for 45 min at room temperature in the dark. Trypsin digestion used protein-to-enzyme ratios of 1:50 and 1:100. Peptides were desalted by solid-phase extraction, separated on a NanoElute UHPLC system, and analysed on a TIMS-TOF Pro mass spectrometer (Bruker, Germany).

2.3 Bioinformatic analysis

Raw MS/MS data were processed with MaxQuant (v.1.6.6.0) against the UniProt human database (HomoSapiens_161584_20180123). We defined differentially expressed proteins as those with fold change > 1.5 or < 0.67 and $P < 0.05$. GO and KEGG enrichment were tested by hypergeometric tests. PPI networks were built in STRING (https://string-db.org/) at confidence score > 0.7. We ranked candidate markers by combining differential expression, pathway enrichment, and network centrality.

2.4 ELISA

ELISA kits were from Mibio (Shanghai, China). In brief, 10 µL urine, 40 µL sample diluent, and 100 µL horseradish peroxidase-conjugated antibody were added to coated wells and incubated for 1 h at 37°C. Wells were washed five times and incubated with 100 µL chromogenic substrate for 20 min at 37°C. After 50 µL stop solution was added, absorbance was read at 450 nm on a microplate reader (BioTek Epoch, Winooski, VT, USA).

2.5 Statistical analysis

Statistics were done with GraphPad Prism 8.01. Normality was tested with the Kolmogorov–Smirnov test. Non-parametric data are given as median [interquartile range] and parametric data as mean ± SD. We used parametric or non-parametric tests as appropriate, with $P < 0.05$ taken as significant. ROC curves and multivariable logistic regression with eGFR as a covariate assessed the diagnostic performance of urinary CTSA.

3 Results

3.1 Clinical characteristics of study participants

Table 1 lists the clinical features of the 20 participants (10 HF, 10 controls) analysed by 4D-LFQ and the 88 participants (48 HF, 40 controls) analysed by ELISA. HF patients had lower LVEF (40.4% ± 16.5% vs 65.6% ± 8.36%, $P = 0.001$ in the proteomics subset; 30.5% [27–35.75] vs 66.5% [61.25–71], $P = 0.003$ in the ELISA subset) and higher NT-proBNP. Serum creatinine was higher (115.57 ± 22.57 vs 65.22 ± 9.47 µmol/L, $P < 0.0001$) and eGFR lower (56.39 ± 13.79 vs 96.5 ± 19.63 mL/min, $P < 0.0001$) in HF patients. All HF patients received aldosterone antagonists and loop diuretics; 70% also received beta-blockers and an ARNI, and 20% an ACEI/ARB.

3.2 Differential protein expression identified by urine proteomics

Figure 1 shows the study workflow. 4D-LFQ identified 11,956 unique peptides and 2047 proteins, of which 1477 were quantified (Fig. 2A). Principal component analysis, relative average deviation, and Pearson correlation confirmed reproducibility (Fig. 2B–D). The volcano plot showed 200 differentially expressed proteins, 82 up- and 118 downregulated in HF (fold change > 1.5, $P < 0.05$; Fig. 3A). GO analysis placed the upregulated proteins in regulation of exocytosis, neutrophil activation, and neutrophil-mediated immunity (molecular functions: hydrolase activity; cellular components:

extracellular region and endomembrane system; Fig. 3B–D). KEGG analysis highlighted the lysosome (map04142), cell adhesion molecules (map04514), apoptosis (map04210), and the renin–angiotensin system (map04614) (Fig. 4A). Of the 25 proteins screened (17 up-, 8 downregulated; Table 2, Fig. 4B), CTSA stood out because it acts in the renin–angiotensin system, which is involved in HF (Fig. 5A). CTSA is a ≈ 52 kDa protein with 32 kDa and 20 kDa domains joined by two disulfide bonds (Fig. 5B).

3.3 Bioinformatics and protein–protein interaction analysis

At STRING confidence > 0.7, the 25 proteins formed a network in which the 17 upregulated ones clustered tightly (Fig. 6A, B). CTSA sat at the centre of this cluster. Its nearest neighbours were mostly lysosomal hydrolases and extracellular peptidases that share CTSA's location and biology: the cathepsins CTSC, CTSZ and CTSH; the glycosidases HEXA, HEXB, MAN2B1, GNS, GLB1, FUCA1 and FUCA2; the peptidases DPP4, ANPEP, ENPEP and PRCP; and the angiotensin precursor AGT. This pattern fits coordinated lysosomal turnover and peptide processing more than isolated upregulation of one enzyme.

Two features of the network matter for HF. First, CTSA is known to stabilise neuraminidase-1 and β-galactosidase in the lysosome and to process renin–angiotensin peptides [16, 17, 19]. AGT clusters with these peptidases, which suggests a local angiotensin-processing axis that includes CTSA. Second, the network agrees with the KEGG results: the lysosome (map04142) and the renin–angiotensin system (map04614) were both strongly enriched. Because a CTSA-centred cluster, lysosomal enrichment, and renin–angiotensin signalling all pointed the same way, we chose CTSA for ELISA validation.

Taken together, the PPI network suggests that urinary CTSA rises not alone but as part of a coordinated lysosomal and peptidase response, which supports its interpretation as a marker linked to cardiac stress.

3.4 Urinary CTSA as a non-invasive biomarker for heart failure

Urinary CTSA was higher in HF than in controls (median [IQR] 3055.43 [2498.33–3434.76] vs 2551.4 [2084.76–2785.9] pg/mL, $P < 0.0001$; Fig. 6C). When divided by serum creatinine, the ratio was lower in HF (31.67 [21.54–40.26] vs 37.72 [27.94–49.10], $P = 0.0088$; Fig. 6D). Serum creatinine in HF is affected by renal venous congestion, volume status, and muscle mass, so creatinine-based normalization will hide the true CTSA signal. Dividing by eGFR (CKD-EPI) gave a ratio that was higher in HF ($P < 0.0001$; Fig. 6E). ROC analysis of the CTSA/eGFR ratio gave an AUC of 0.89 (95% CI 0.83–0.96, $P < 0.0001$); the best cut-off, 30.44, yielded 85.0% sensitivity and 81.25% specificity (Fig. 6F). In logistic regression adjusted for eGFR, the CTSA–HF link stayed positive ($\beta = 524.098$, $P = 0.001$). Creatinine-based normalization therefore distorts the urinary CTSA readout in HF, and renal-function adjustment is needed for correct interpretation.

4 Tables

Table 1
Clinical characteristics of study participants

Characteristics	HF (n = 10)	Control (n = 10)	P	HF (n = 48)	Control (n = 40)	P
Age, year	68.6 ± 8.76	58.3 ± 13.04	0.032	65.3 ± 13.31	59.3 ± 12.03	0.053
BMI (body mass index), kg/m2	22.07 ± 2.53	22.51 ± 2.04	0.554	24.33 ± 3.67	23.87 ± 3.54	0.679
Male sex, n (%)	9(90)	2(20)	0.826	30(62.5)	24(60)	0.005
SBP (systolic blood pressure), mmHg	108.1 ± 15.98	133.2 ± 13.64	0.002	109.5 (95.5-122.4)	124.5 (110.5-139.5)	0.001
DBP (diastolic blood pressure), mmHg	69.5 (62-77.5)	73.5 (64.25-80)	0.007	69.85 ± 13.08	76.95 ± 10.78	0.448
HR (heart rate), bpm	84.2 ± 22.17	75.4 ± 11.93	0.312	75.5 (64.25-86)	75 (61.5–79)	0.288
NYHA class II/III/IV, n (New York Heart Association)	1/2/7	/	/	6/15/27	/	/
LVEF (left ventricular ejection fraction), %	40.4 ± 16.5	65.6 ± 8.36	0.001	30.5 (27-35.75)	66.5 (61.25-71)	0.003
LVESD (left ventricular end-systolic dimension), mm	45 (33-55.5)	31 (27–34)	< 0.0001	60.5 (56.25–65.75)	32 (28–34)	0.024
LVEDD (left ventricular end- diastolic dimension), mm	63.2 ± 12.56	48.8 ± 4.32	< 0.0001	72.35 ± 9.27	50.5 ± 4.95	0.006
LA (left atrium), mm	51.5 (47.75–62.75)	34 (34-39.5)	< 0.0001	51.33 ± 7.85	37.58 ± 5.32	0.001
Hypertension	6 (60)	6 (60)	0.367	14 (29.2)	16 (40)	1.0
CHD (cornary heart disease)	3 (30)	1 (10)	1.0	9 (18.8)	7 (17.5)	0.582
Diabetes mellitus	3 (30)	0 (0)	0.561	9 (18.8)	5 (12.5)	0.211
AF (atrial fibrillation)	5 (50)	3 (30)	0.064	19 (39.6)	8 (20)	0.65
Stroke	3 (30)	3 (30)	0.397	10 (20.8)	5 (12.5)	1.0

Characteristics	HF (n = 10)	Control (n = 10)	P	HF (n = 48)	Control (n = 40)	P
CRI (chronic renal insufficiency)	4 (40)	0 (0)	0.001	11 (22.9)	0 (0)	0.087
NT-proBNP, pg/mL (N-terminal pro brain natriuretic peptide)	2870 (1035-9265.75)	167.5 (21-393.75)	< 0.0001	3504.5 (2046.25-12448.25)	50 (50-161.25)	< 0.0001
SCr, μmol/L ()	115.57 ± 22.57	65.22 ± 9.47	< 0.0001	92.5 (79.25–121)	64 (53.5-78.25)	< 0.0001
BUN, mmol/L (blood urea nitrogen)	12.22 ± 3.11	5.79 ± 1.22	< 0.0001	8.085 (6.43–10.75)	5.78 (4.7–7.1)	< 0.0001
GFR, mL/min ()	56.39 ± 13.79	96.5 ± 19.63	< 0.0001	67.35 (49.55–80.77)	110.32 (88.42-124.19)	< 0.0001
TC, mmol/L (total cholesterol)	2.71 (2.28–3.42)	4.23 (3.38–5.33)	0.012	4.07 ± 0.99	4.62 ± 1.0	0.017
TG, mmol/L (triglyceride)	0.86 (0.56–1.56)	1.34 (1.11–2.06)	0.217	1.16 (0.9–1.55)	1.38 (0.95–1.95)	0.096
HDL (high-density lipoprotein), mmol/L	0.94 ± 0.18	1.09 ± 0.23	< 0.0001	0.86 (0.73–1.03)	1.11 (0.92–1.3)	0.123
LDL-C (low density lipoprotein cholesterol), mmol/L	1.47 (0.82–1.83)	2.41 (1.44–3.23)	0.262	2.32 ± 0.68	2.51 ± 0.84	0.075
VLDL (very low-density lipoprotein), mmol/L	0.72 ± 0.18	0.85 ± 0.24	0.287	0.78 (0.67–0.95)	0.88 (0.66–1.05)	0.205
Hemoglobin, g/L	122.2 ± 21.24	126.2 ± 11.97	0.86	126.65 ± 20.06	130.48 ± 17.49	0.61
Medication, n (%)						
ACEI/ARB (angiotensin-converting enzyme Inhibitor/angiotensin receptor blocker)	2 (20)	2 (20)	1.0	1 (2.1)	0 (0)	1.0
Beta-blocker	7 (70)	1 (10)	< 0.0001	40 (83.3)	4 (10)	0.02
Aldosterone antagonist	10 (100)	0 (0)	< 0.0001	41 (85.4)	0 (0)	< 0.0001

Characteristics	HF (n = 10)	Control (n = 10)	P	HF (n = 48)	Control (n = 40)	P
Loop diuretic	10 (100)	0 (0)	< 0.0001	46 (95.8)	0 (0)	< 0.0001
ARNI (Angiotensin receptor enkephalinase inhibitor)	7 (70)	0 (0)	< 0.0001	41 (85.4)	2 (5)	0.003

Table 2
Differentially expressed proteins in the urine of patients with heart failure and healthy controls

Accession	Gene name	Protein name	Expression	Fold change	p
P04066	FUCA1	Tissue alpha-L-fucosidase	Up	3.0665	< 0.0001
Q9BTY2	FUCA2	Plasma alpha-L-fucosidase	Up	2.6364	0.0045
P07686	HEXB	Beta-hexosaminidase subunit beta	Up	2.6292	0.0026
Q07075	ENPEP	Glutamyl aminopeptidase	Up	2.496	0.0109
P53634	CTSC	Dipeptidyl peptidase	Up	2.4113	0.0005
Q9UBR2	CTSZ	Cathepsin Z	Up	2.3538	0.0006
P27487	DPP4	Dipeptidyl peptidase 4	Up	2.3377	0.0008
P15586	GNS	N-acetylglucosamine-6-sulfatase	Up	2.3067	0.0007
O00754	MAN2B1	Lysosomal alpha-mannosidase	Up	2.2735	0.0013
P06865	HEXA	Beta-hexosaminidase subunit alpha	Up	2.1697	0.0029
P01019	AGT	Angiotensinogen	Up	2.1024	0.0126
P09668	CTSH	Pro-cathepsin H	Up	2.0339	0.0069
P15144	ANPEP	Aminopeptidase N	Up	1.9121	0.0054
P10619	CTSA	Lysosomal protective protein	Up	1.8987	0.0018
Q8NCC3	PLA2G15	Phospholipase	Up	1.7353	0.0193
P42785	PRCP	Lysosomal Pro-X carboxypeptidase	Up	1.6708	0.0267
P16278	GLB1	Beta-galactosidase	Up	1.5026	0.0241
P01133	EGF	Pro-epidermal growth factor	Down	0.3413	0.0003
P54753	EPHB3	Ephrin type-B receptor 3	Down	0.4982	0.0008
P32004	L1CAM	Neural cell adhesion molecule L1	Down	0.5536	0.0024
Q12860	CNTN1	Contactin-1	Down	0.5646	0.0005
Q92896	GLG1	Golgi apparatus protein 1	Down	0.5681	0.0079
P19022	CDH2	Cadherin-2	Down	0.5752	0.0234

Accession	Gene name	Protein name	Expression	Fold change	p
O15197	EPHB6	Ephrin type-B receptor 6	Down	0.5889	0.0064
P29323	EPHB2	Ephrin type-B receptor 2	Down	0.5976	0.0275

4 Discussion

Urine is an early and sensitive source of biomarkers. Blood changes are buffered by homeostasis, but urine accumulates systemic alterations and can reveal disease processes early [7]. Urine composition is still affected by age, sex, and diet [8], which animal models help control [8–11]. Clinical urine-proteomics studies have found markers for gastric cancer [12], familial Parkinson's disease [13], and carotid artery stenosis [14], and the method is now used for early diagnosis and monitoring [15]. In dilated cardiomyopathy, Su et al. reported plasma proteome changes and proposed carbonic anhydrase 2 and 3 as diagnostic candidates [15].

In this study, urinary CTSA was higher in HF, which supports its use as a non-invasive urinary marker. We combined 4D-LFQ proteomics with ELISA to move from an open screen to a targeted measurement, and bioinformatics picked CTSA out of 200 differentially expressed proteins. ELISA confirmed the rise and showed that the CTSA/eGFR ratio separates HF from controls with an AUC of 0.89.

CTSA is a lysosomal glycoprotein with deamidase, esterase, and carboxypeptidase activities, and it is strongly expressed in kidney, lung, liver, placenta, and heart [16]. In the lysosome it forms a complex with neuraminidase-1 and β-galactosidase that protects these partners from proteolysis [16, 17]. It shows deamidase and esterase activity at neutral pH and serine carboxypeptidase activity at acidic pH [16, 17]. Loss of CTSA leads to lysosomal buildup of sialylated oligosaccharides and glycoproteins, which are then excreted [17, 18]. CTSA also breaks down extracellular peptides such as endothelin-1, bradykinin, and angiotensin I [19] and takes part in the renin–angiotensin system by converting angiotensin I to angiotensin II in the human heart [19]. Through this route it affects blood pressure and fluid balance.

The PPI network supports this view. CTSA sat at the hub of a cluster of co-expressed lysosomal hydrolases and peptidases, which indicates that urinary CTSA rises as part of a coordinated lysosomal response rather than on its own. AGT, the renin–angiotensin precursor, sat in the same cluster. This agrees with CTSA's known role in angiotensin processing and links the urinary signal to a pathway already tied to HF.

CTSA has drawn interest as a treatment target in HF. In models of myocardial infarction, type 2 diabetes, and angiotensin-II-induced hypertrophy, cardiac CTSA rises, and blocking it protects the heart, limits hypertrophy, and reduces fibrosis [20–22]. Petrera et al. found that CTSA inhibition lowered plasma BNP and reduced stress-response proteins such as periostin, troponin T, and cytoglobin [22].

The CTSA/creatinine ratio was lower in HF even though absolute CTSA was higher. This exposes a confounder: serum creatinine, a neutral reference in health, is altered in HF by renal venous congestion, fluid status, and muscle loss. These factors uncouple creatinine from true filtration and artificially lower the ratio. After eGFR adjustment, which corrects for age, sex, and muscle mass, the association became positive. This suggests that the higher urinary CTSA reflects active release linked to myocardial stress rather than creatinine kinetics. Creatinine-based normalization is therefore misleading for urinary biomarkers in HF; eGFR or cystatin C should be used instead.

5 Limitations

It was single-center and included a modest number of HF patients, treated as one group that may not cover all HF phenotypes. The CTSA finding needs validation in an independent cohort, and its added value over established markers such as NT-proBNP has not been tested head-to-head. Other proteins among the 200 candidates could work with CTSA to improve diagnosis. Lastly, the PPI interpretation rests on an in-silico network and should be confirmed experimentally.

6 Conclusion

This study shows that urinary CTSA is raised in heart failure and, when read with renal function, acts as a non-invasive diagnostic marker (AUC 0.89). The link held after eGFR adjustment, which avoids the confounding seen with creatinine-based normalization. Together with the PPI evidence of coordinated lysosomal activation, these findings support urinary CTSA—interpreted alongside renal function—as an addition to blood-based tests for HF diagnosis and monitoring.

Declarations

Ethics approval and consent to participate.

The study was approved by the Institutional Review Board of [Medical Research Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China] (protocol AF/SC-14/05.0, April 9,2025) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent.

Competing interests.

The authors declare no competing interests.

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Author Contribution

Conceptualization, A.Z. and D.C.; methodology, K.H.; software, A.Z. and Y.H.; validation, M.W.; formal analysis, A.Z.; investigation, D.C.; resources, A.Z. and D.C.; data curation, A.Z. and W.C.; writing—original draft, A.Z.; writing—review and editing, W.C. and H.S.; visualization, A.Z.; supervision, H.S.; project administration, H.S.; funding acquisition, H.S.

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During the preparation of this study, the author used https://www.uniprot.org/ for display the crystal structure of urinary lysosomal cathepsin.

Availability of data and materials.

The processed protein quantification data supporting this study are openly available in the ScienceDB repository at https://www.scidb.cn/s/aYbYja under a CC BY 4.0 license.

Abbreviations

HF Heart failure

GO Gene Ontology

KEGG Kyoto Encyclopedia of Genes and Genomes

PPI Protein–protein interaction

CTSA Lysosomal cathepsin A

LVEF Left ventricular ejection fraction

NYHA New York Heart Association

eGFR Estimated glomerular filtration rate

ELISA Enzyme-linked immunosorbent assay

4D-LFQ 4D label-free quantitative proteomics

NT-proBNP N-terminal pro b-type natriuretic peptide

ARNI Angiotensin receptor neprilysin inhibitor

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Figures

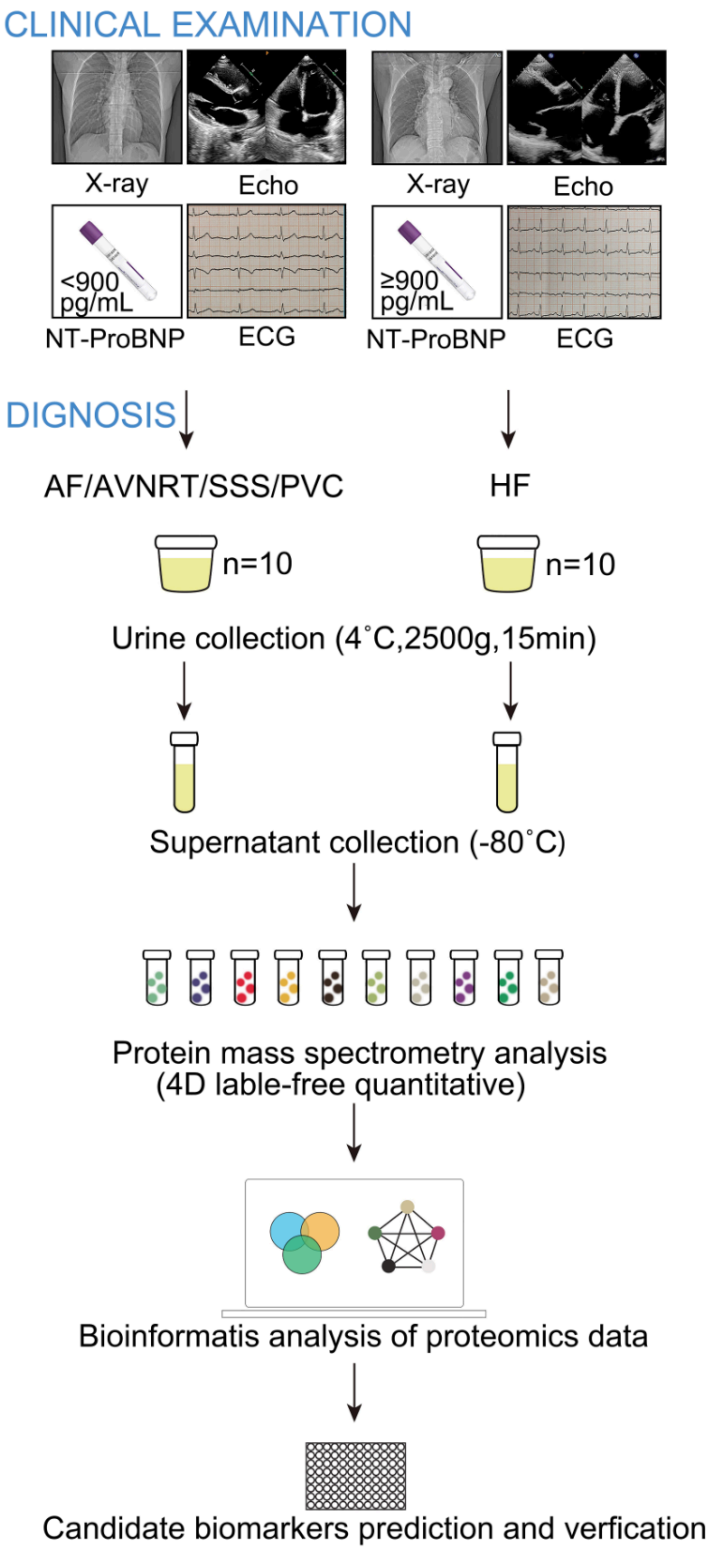


Figure 1

Urinary proteome study in patients with HF and NCs. Workflow for urine proteome profile analysis and validation of HF biomarkers. UCG: ultrasonic cardiogram; ECG: electrocardiogram; HF: heart failure; NC: normal control.

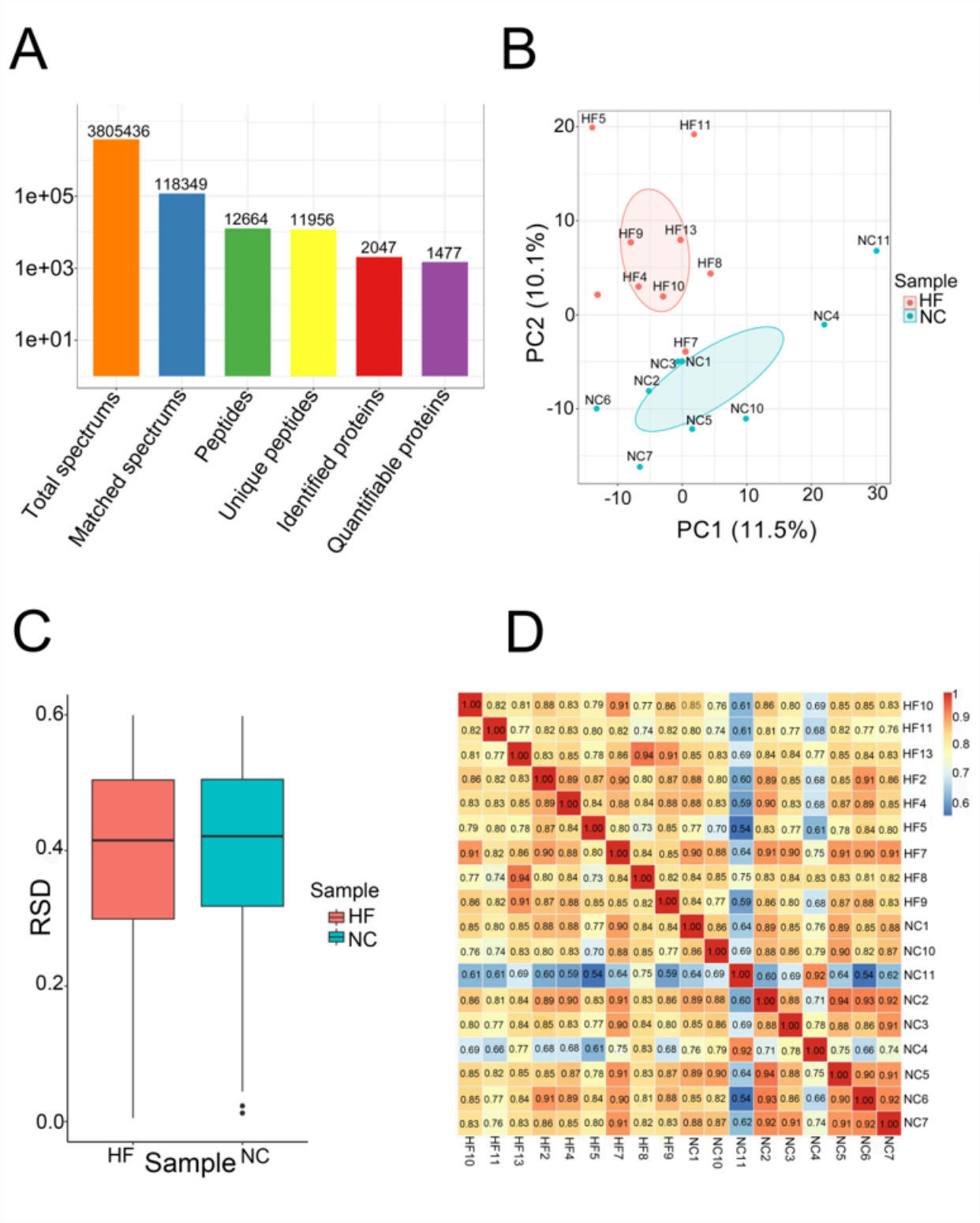


Figure 2

Urinary proteome study in patients with HF and NCs. (A) Quantification overview of peptides and proteins identified by mass spectrometry. Total spectrum: number of secondary spectra produced. Matched spectra: number matching the theoretical secondary spectrum. Peptides: total peptide sequences matched. Unique peptides: distinct peptide sequences. Quantifiable proteins: those counted using specific peptides. (B–D) Evaluation of the quantitative repeatability of protein.

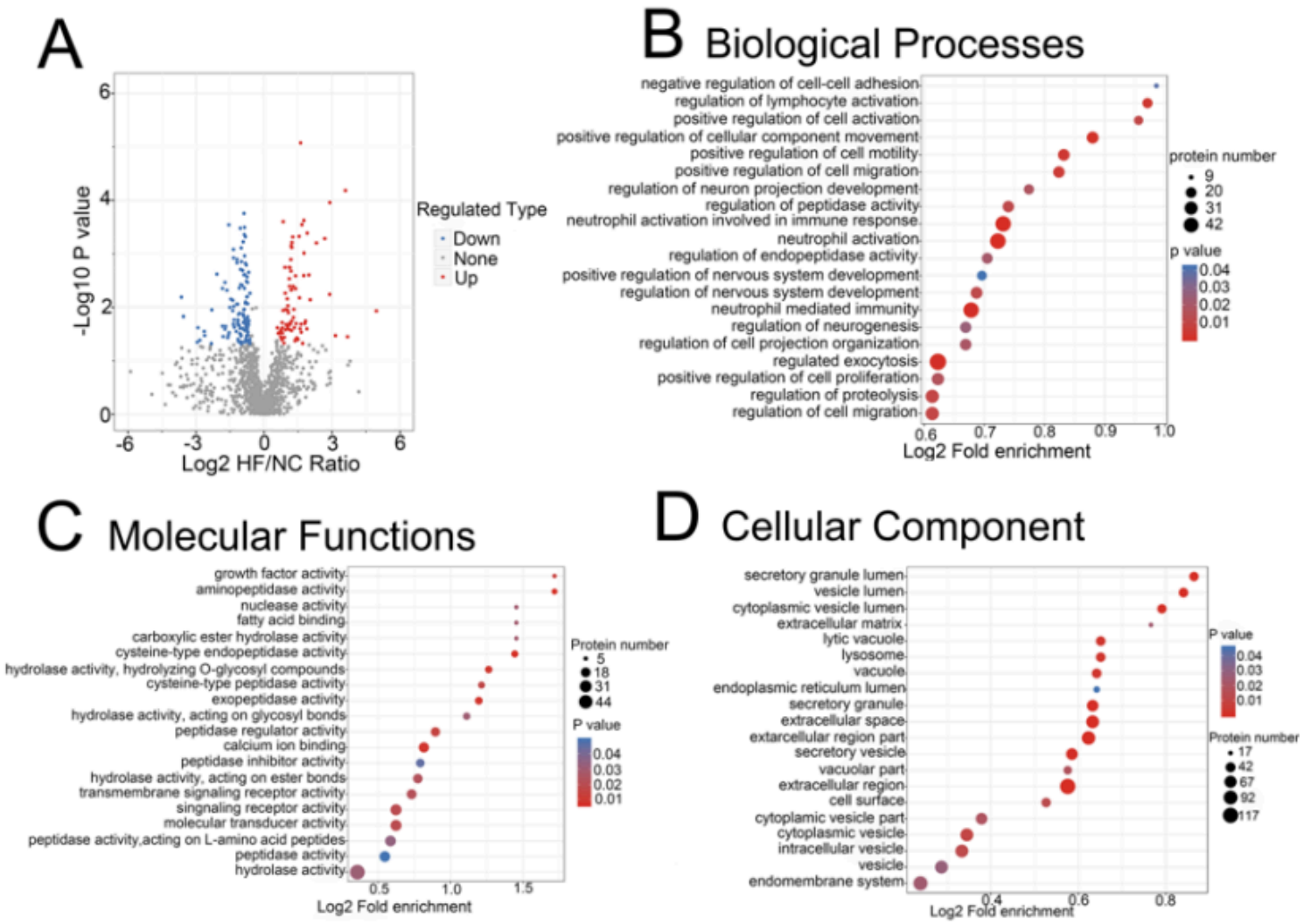


Figure 3

GO enrichment analyses for significantly differentially expressed proteins. (A) Volcano plot showing differentially expressed proteins between HF patients and normal controls. Red dots: upregulation; blue dots: downregulation. (B) Biological process; (C) Molecular function; (D) Cellular component.

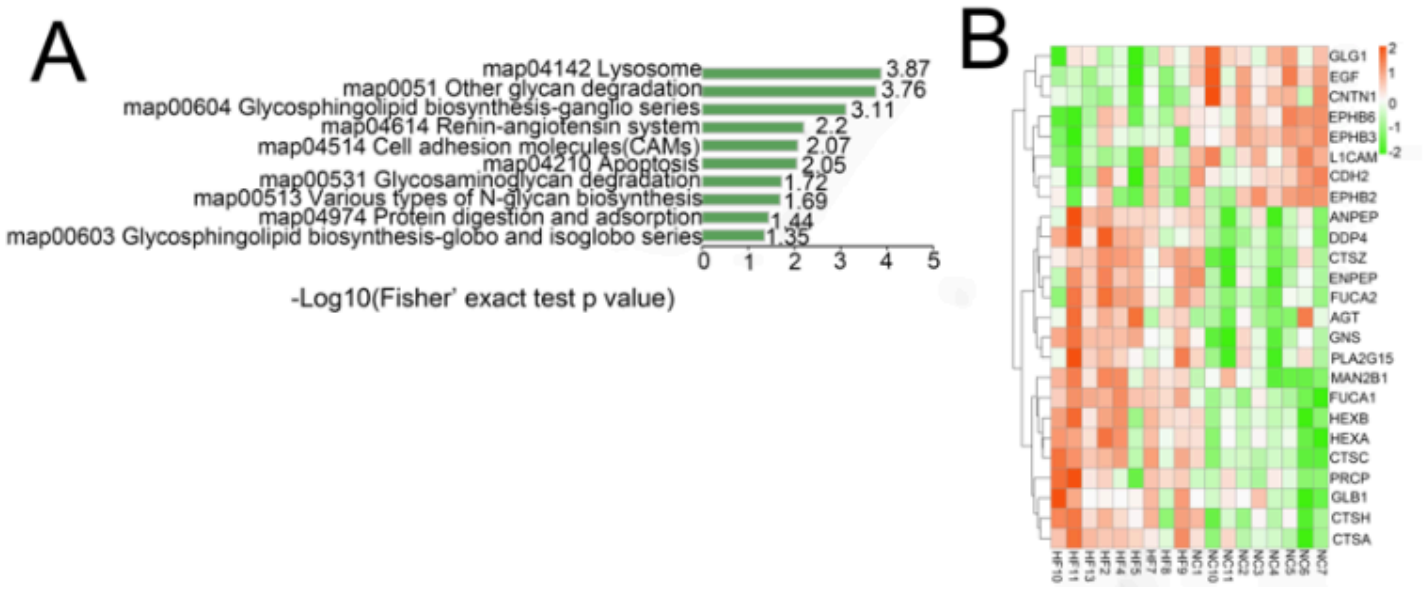


Figure 4

KEGG pathway enrichment analyses for significantly differentially expressed proteins. (A) KEGG pathway analysis reveals enhanced pathways in the urine of HF patients. (B) Fold-change of the top 17 upregulated and top 8 downregulated proteins, indicated by orange and green, respectively.

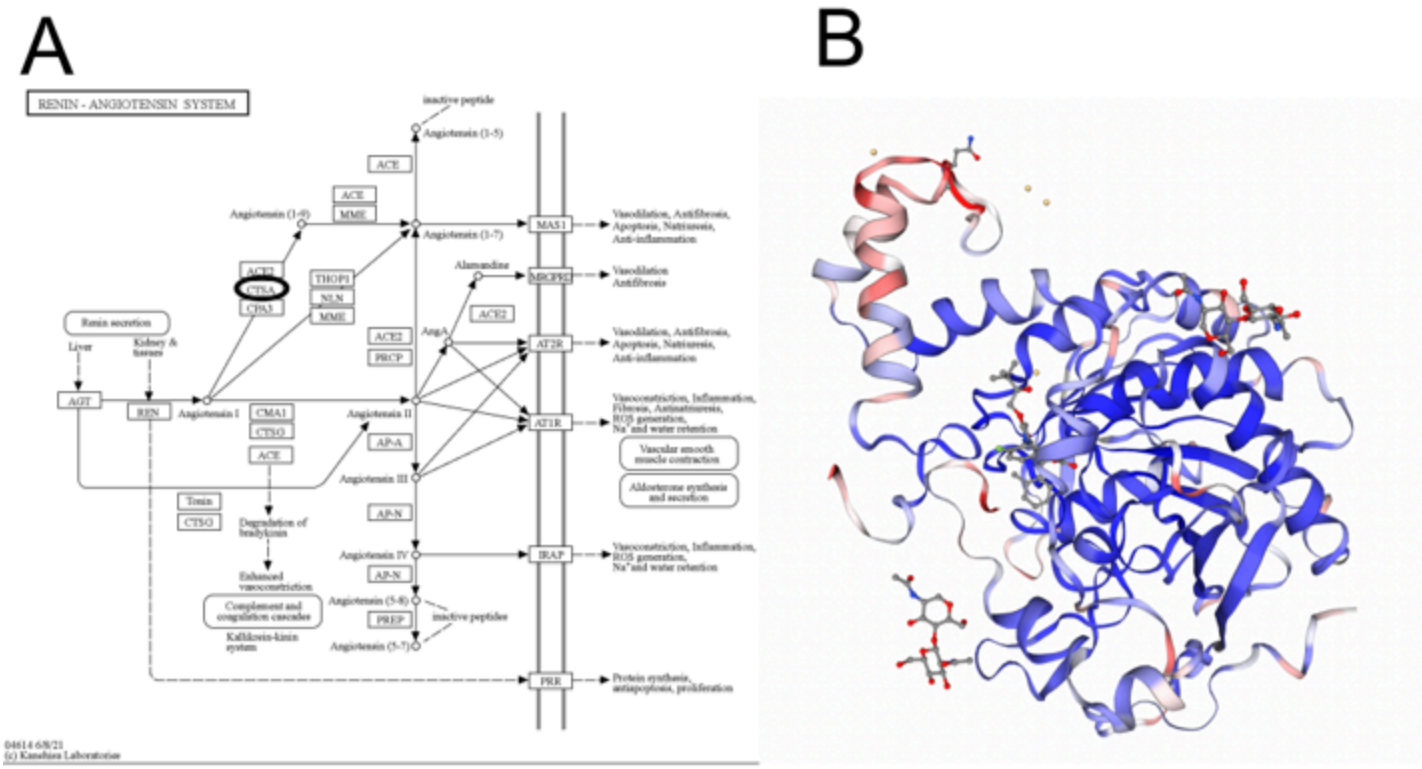


Figure 5

The renin–angiotensin system (map04614) signalling pathway and crystal structure of urinary lysosomal cathepsin. (A) The renin–angiotensin system (map04614) pathway, in which cathepsin A participates, obtained from the global proteome data by KEGG analysis. (B) Crystal structure of urinary lysosomal cathepsin, showing sequence regions, domains, known secondary structural elements, and predicted intrinsic flexibility.

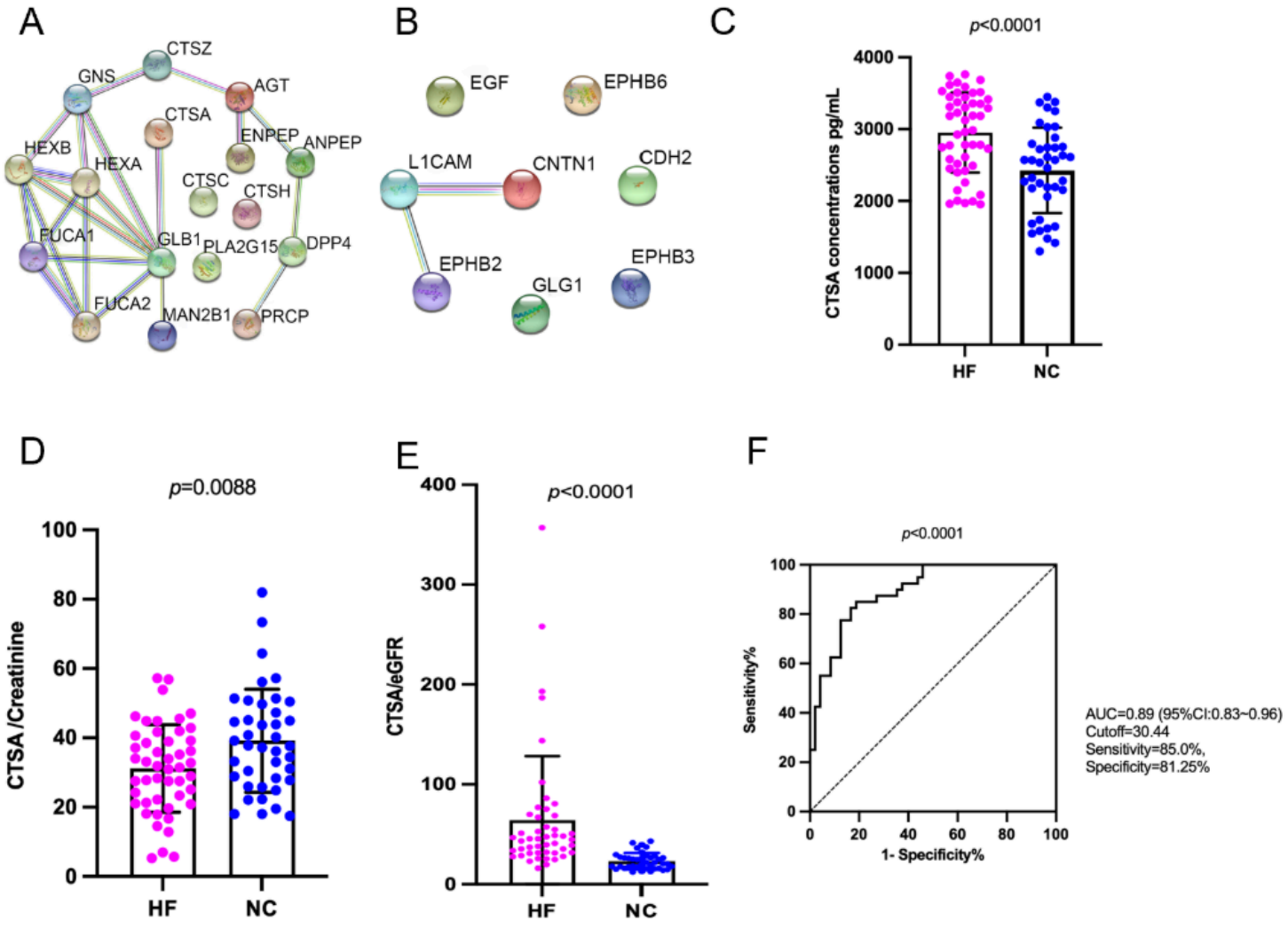


Figure 6

Urinary CTSA as a non-invasive biomarker for heart failure. (A–B) Protein–protein interaction networks of differentially expressed urinary proteins. (C) Urinary CTSA concentrations in 48 HF patients and 40 normal controls (NC). (D) Ratio of urinary CTSA to creatinine in HF and NC. (E) Ratio of urinary CTSA to eGFR in HF and NC. (F) ROC curve of the CTSA/eGFR ratio for discriminating HF from NC. AUC, area under the curve.