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Research article

# Screening of serum markers in patients with resistant hypertension

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ABSTRACT

*Background:* This study delves into the intricacies of resistant hypertension (RH), a prevalent yet enigmatic chronic cardiovascular ailment that is linked to a myriad of complications. Although its full pathogenesis is still shrouded in mystery, the field of proteomics offers a beacon of hope, with its potential to shed light on the proteins that orchestrate the tapestry of life. Harnessing the power of proteomics is essential for demystifying the pathogenesis of RH, enabling more precise diagnostics and treatments, and ultimately improving prognostic outcomes.

*Methods:* Our approach was to employ rigorous statistical analyses to home in on proteins with significant expression variances between our two cohorts. We complemented this with bioinformatics tools to unravel the intricate functions and pathways of these proteins. By synthesizing these insights with the clinical profiles of our patients, we were able to distill a set of definitive biomarkers with diagnostic potential. In our quest for clarity, we also embarked on a retrospective journey, amassing and scrutinizing clinical data from both RH and hypertension (HTN) patients. We crafted and rigorously assessed risk factor models to evaluate their diagnostic prowess.

*Results:* Our exploration spanned across 30 blood samples from RH patients and 20 from those grappling with HTN. Our inquiry yielded some compelling revelations: (1) RH patients showcased 29 unique proteins, in contrast to the 59 unique proteins found in HTN patients. A deeper dive into the proteomic data unveiled molecular functions predominantly tied to lipid metabolism, protein networking, and oxidative stress, with a spotlight on pathways such as cholesterol metabolism, coagulation, and the complement cascade. (2) By charting receiver operating characteristic curves and rigorously analyzing the proteomic data, we surfaced 11 proteins with notable diagnostic potential, tightly interwoven with clinical metrics.

*Conclusion:* Our research has pinpointed 11 proteins that stand as promising serum biomarkers, endowed with significant diagnostic value. This discovery marks a stride towards a more nuanced understanding and management of resistant hypertension.

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## 1. Introduction

The "China Cardiovascular Health and Disease Report 2020" highlights the profound challenge of cardiovascular disease (CVD) to the health of the Chinese population. The number of individuals currently suffering from CVD has reached a daunting 330 million, cementing its status as an urgent health issue [1]. Within this context, hypertension stands out as a common manifestation of CVD, accounting for roughly 74 % of the affected population, which translates to around 245 million people [1]. Among those with hypertension, a significant proportion—about 20%—struggle with resistant hypertension (RH) [2]. RH is defined by the persistent elevation of blood pressure even when patients are taking three different classes of antihypertensive drugs at their maximum doses for at least a month, or when more than four such medications are required alongside lifestyle changes to reach the desired blood pressure goals [3]. This stubbornly hypertension poses a serious threat to the health and quality of life of those affected. Studies show that individuals with RH are at a doubled risk for cardiovascular events compared to those with normal blood pressure [4]. Furthermore, RH is often accompanied by significant damage to vital organs [5]. Current understanding suggests that the development of RH is closely linked to factors such as increased retention of sodium and water, overactivity of the renin-angiotensin-aldosterone system, heightened activity of the sympathetic nervous system, increased arterial stiffness, and imbalances in gut microbiota [6]. However, a complete understanding of these mechanisms in RH patients is still lacking, calling for immediate and in-depth scientific research.

Proteomics is a cutting-edge scientific tool that allows for the exact measurement of proteins present in cells, tissues, or body fluids, thus revealing their essential function in the processes of life [7]. With its exceptional sensitivity and specificity, this technology is of great importance for understanding the causes of diseases, aiding in early detection, developing new medications, and forecasting the course of diseases [8]. This has led to a notable increase in interest within the field of cardiovascular research.

Prominent researchers, such as Gajjala and colleagues [9], have meticulously studied individuals with hypertension (HTN) and compared them to healthy individuals, identifying key proteins that are central to the development and treatment of HTN. This groundbreaking research opens new avenues for the study and treatment of HTN. Similarly, the work led by Liu Zunzhong and his team [10] has highlighted that the proteins that differ in heart disease caused by HTN are primarily involved in vital processes like oxidative stress and energy metabolism. Moreover, Liu Shengze and his colleagues [11] have identified crucial biomolecular markers linked to cerebral hemorrhage due to HTN through a detailed analysis of proteomics in the cerebrospinal fluid of affected individuals. In addition, Tingting Liu and her research group [12] found an increased and specific expression of the Rap1 protein in their investigation of renal damage in HTN, shedding new light on potential pathways for a deeper understanding of renal damage in HTN.

Despite significant progress in serum proteomics for HTN patients, the exploration of proteomics in RH is still in its infancy, especially regarding the identification of serum markers. There is a pressing need to conduct comprehensive serum proteomic studies on RH patients to gain a better understanding of its underlying mechanisms and potential therapeutic targets. Our principal goal was to distinctly categorize patients into two groups based on their hypertension profiles: the RH group and the HTN group. Such an endeavor could provide new insights and approaches for the diagnosis and treatment of RH, ultimately improving the quality of life and outcomes for patients.

## 2. Methods

### *2.1. Subjects and sample acquisition*

#### *2.1.1. Selection parameters*

1) Criteria for Mass Spectrometry Analysis Sample Selection

**Inclusion Criteria: RH Inclusion Criteria:**① Following a 4-week treatment regimen with a minimum of three antihypertensive medications (including one diuretic) at optimal dosages, blood pressure levels remained higher than the target threshold; or four antihypertensive drugs were necessitated to achieve the target level; ② Age between 18 and 85 years; ③ Patients provided informed consent and willingly participated in the study.

**Inclusion Criteria for the HTN Group:**① Office systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg without antihypertensive medication; ② Previously diagnosed with HTN or currently under antihypertensive medication; ③ Non-RH patients; ④ Patients who signed an informed consent form and voluntarily participated in this study.

Exclusion Criteria: ① Severe cardiac, hepatic, and renal insufficiency; ② Patients with acute HTN and secondary HTN; ③ Pregnant and lactating women; ④ Acute stage of infection; ⑤ Tumor, immune system, hematopoietic system diseases.

2) Clinical Analysis Data Screening Criteria

**Inclusion Criteria:** ①Having at least two or more hospitalization medical records; ② Interval between the two medical record entries exceeding 4 weeks, demonstrating the administration of three (at least one being a diuretic) or more antihypertensive drugs; ③ Hospitalized patients receiving three antihypertensive drugs with monitored blood pressure, SBP>140/DBP>90 mmHg.

**Exclusion Criteria**①Severe cardiac, hepatic, and renal insufficiency; ② Patients with acute HTN and secondary HTN; ③ Pregnant and lactating women; ④ Acute stage of infection; ⑤ Tumor, immune system, hematopoietic system diseases.

#### 2.1.2. Sample size determination

We initiated a pre-test in accordance with our experimental design, yielding the subsequent results: patients were meticulously matched based on their age. For the mass spectrometry analysis, we selected 5 cases each of patients with RH and those with HTN. The mass spectrometry outcomes revealed an effect value of 0.65 for both samples. Employing the formula: $*N = [2*(Z\alpha / 2 + Z)\ \beta\ 2\ *\sigma 2\ ]/\delta 2*$, we calculated N to be 19. Hence, we determined that a minimum of 19 serum samples were required for inclusion in both the RH and HTN groups.

#### 2.1.3. Data and sample collection

We meticulously gathered comprehensive patient data, ensuring a thorough understanding of each individual's health profile. Fasting blood samples were collected with the utmost care, adhering to ethical standards and ensuring patient consent.

### 2.2. Biochemical and clinical assessments

#### 2.2.1. Biochemical indicator detection methods

Biochemical indicators encompassing liver function, kidney function, blood lipids, and blood glucose were detected using the enzyme endpoint colorimetric assay. The assay employed kits supplied by Roche and was conducted on a Roche Cobas 8000 biochemical autoanalyzer.

#### 2.2.2. Ultrasonic data detection methods

Cardiac ultrasound examinations utilized a Philips EPIQ model 7C (Philips, The Netherlands) color Doppler ultrasound diagnostic machine equipped with an X5-1 probe operating at a frequency range of 1–5 MHz. Patients were positioned either in the left lateral or flat position.

### 2.3. Proteomic analysis

#### 2.3.1. Sample processing

The protocol comprised of: ① high abundance protein elimination; ② determination of protein concentration; ③sample digestion; and ④peptide desalination.

#### 2.3.2. Database searching and data processing

Mass spectrometry data were input into Proteome Discoverer 2.4 (Thermo Fisher, USA) software for qualitative analysis, with mass spectra screened and searched within the Uniprot protein database for human sapiens.

#### 2.3.3. Screening for differential proteins

① Bioinformatics Analysis
- **Volcano Plotting** Statistical analysis of the obtained protein data was performed, calculating fold change (FC), logarithmized fold change (log FC), and significance level (P) differences between the sample groups. Volcano plots were generated based on these results. Proteins meeting the criteria of log FC $> 1.5$ and $P < 0.05$ were deemed up-regulated, while those meeting log FC $< -1.5$ and $P < 0.05$ were considered down-regulated.
- **Cluster Analysis** Utilizing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, cluster analysis was executed.

② Differential Protein Screening
- **Characteristic Curves for Subjects** Receiver Operating Characteristic (ROC) curves were separately plotted for up-regulated and down-regulated proteins, evaluating their diagnostic potential by calculating the Area Under Curve (AUC). Proteins meeting criteria of AUC $>0.85$, sensitivity $>85$ %, and specificity $>85$ % were classified as differential proteins.
- **Correlation Analysis Between Clinical Data and Differential Proteins** The screened differential proteins were analyzed for correlation with clinical indicators. During this analysis, proteins meeting the two-tailed significance level (p-value) of less than 0.05 and correlation coefficient greater than 0.3 were considered the final screened significant differential proteins.

③ Clinical Characterization

The clinical data of patients with RH and those with HTN were retrospectively collected and subjected to statistical analysis. Indicators demonstrating statistically significant differences between RH and HTN patients were identified, and a risk factor model was formulated. Subsequently, ROC curve was plotted based on this risk factor model to elucidate the model's diagnostic efficacy.

### 2.4. Data analysis and statistical methods

Data analysis was performed using SPSS 26.0 and R (4.2.1). Missing data $\geq$10 % were deleted and $<$10 % were interpolated using multiple interpolation. Measurement data were presented as Mean $\pm$ SD or utilizing the quartile method. Statistical analysis involved T-test or *U* test. Count data were expressed as percentages and subjected to chi-square testing. ROC curves were employed to assess the diagnostic effects of relevant indicators individually. Correlation analysis was conducted using the Pearson or Spearman method.

Univariate and multivariate logistic regression techniques were utilized to identify risk factors. A visualized logistic Nomograms model was established. $P < 0.05$ were considered to indicate statistical significance.

### 2.5. Research ethics

This study adhered to the ethical guidelines outlined in the Declaration of Helsinki by the World Medical Association, along with norms and regulations pertaining to clinical research. Approval for the study was obtained from the Ethics Committee of Jinan Central Hospital, under Ethics No. 2021-213-01. All participants in the mass spectrometry segment provided informed consent. Clinical data were retrospectively collected from patients and extracted from the medical big data system while safeguarding sensitive information. The results and data involved in this study were not linked to the patients' treatment process, thereby obviating the need for patients to sign an informed consent form.

## 3. Results

### 3.1. Mass spectrometry section results

#### 3.1.1. Comparison of baseline data between RH patients and HTN patients

Analysis of baseline data for the 50 patients included in the proteomic analysis revealed that gender, age, Body Mass Index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), history of smoking, alcohol consumption, coronary artery disease, diabetes mellitus, hyperlipidemia, and the duration of HTN in patients of the RH group showed no statistically significant differences ($P > 0.05$). However, the systolic blood pressure of RH group patients was significantly higher than that of patients with ordinary HTN ($P < 0.05$). This difference was statistically significant, as shown in Table 1.

#### 3.1.2. Proteomic profiles

We compared the proteomic data of RH patients and regular HTN patients separately. The study results indicated that a total of 397 protein expressions overlapped between these two patient groups. RH patients had 29 specific proteins, while HTN patients had 59 specific proteins. Specific data are presented in Fig. 1. Serum proteomics data from RH patients and HTN patients were subjected to GO enrichment analysis (Fig. 2). The results demonstrated that the proteomic data of RH patients primarily encompassed key biological processes such as coagulation, immune response, and metabolic regulation. They involved multiple cellular components including blood components, lipid metabolism, protein transport, and various molecular functional domains such as lipid metabolism, protein interactions, and oxidative stress. On the other hand, GO enrichment analysis of the proteomics data of HTN patients revealed their involvement in important biological processes such as immune response, metabolic regulation, and coagulation. They were associated with cellular components such as intracellular vesicles, membrane surface, and extracellular matrix, and molecular functions such as protein degradation, lipid metabolism, and cellular adhesion. Additionally, KEGG enrichment analysis of serum proteomic data from RH patients and HTN patients showed that the proteomic analysis results of both groups focused on key pathways such as cholesterol metabolism, coagulation, and complement cascade reactions.

#### 3.1.3. Differential protein analysis

*3.1.3.1. Screening for up-regulated and down-regulated proteins.* To explore the proteomic differences between RH patients and HTN patients, we calculated the FC, log FC, and differences in P for the proteins of the two patient groups. This led to the identification of a total of 142 up-regulated proteins and 1 down-regulated protein. Fig. 3.

**Table 1**
The comparison of baseline material.

| | HBP (n = 20) | RH (n = 30) | P value |
|---|---|---|---|
| Sex (male, n%) | 14 (70 %) | 18 (60 %) | 0.34 |
| Age (years) | 60 (46, 64) | 60 (46, 64) | 0.78 |
| BMI (kg/m$^2$) | 25.24 (23.34, 30.07) | 26.21 (25.17, 28.28) | 0.75 |
| SBP (mmHg) | 144 (133, 158) | 166 (145, 181) | 0.02 |
| DBP (mmHg) | 94 (87, 99) | 96 (84, 106) | 0.38 |
| HR (beats/min) | 76 (68, 85) | 77 (72, 88) | 0.42 |
| Smoking (n%) | 4 (20 %) | 6 (20 %) | 0.65 |
| Drinking (n%) | 4 (20 %) | 6 (20 %) | 0.65 |
| CAD (n%) | 6 (30 %) | 8 (27 %) | 0.52 |
| DM (n%) | 4 (20 %) | 8 (27 %) | 0.43 |
| Hyperlipidemia (n%) | 2 (10 %) | 3 (10 %) | 0.69 |
| HBP time (years) | 7.00 (0.65, 13.75) | 7.50 (1.00, 10.00) | 0.97 |

The data are presented as quartile division for non-continuous variables and n for categorical variables. HBP: high blood pressure; RH: resistant hypertension; BMI: body mass index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HR: heart rate; CAD: cardiac artrial disease; DM: diabetes mellitus.

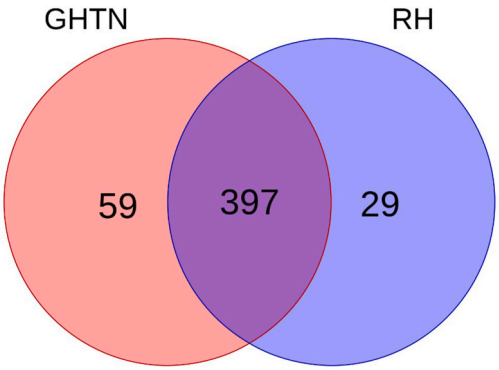


**Fig. 1.** Veen figure. This figure showed that there were 29 specific proteins in RH group and 59 specific proteins in HTN group. RH: resistant hypertension; HTN: hypertension.

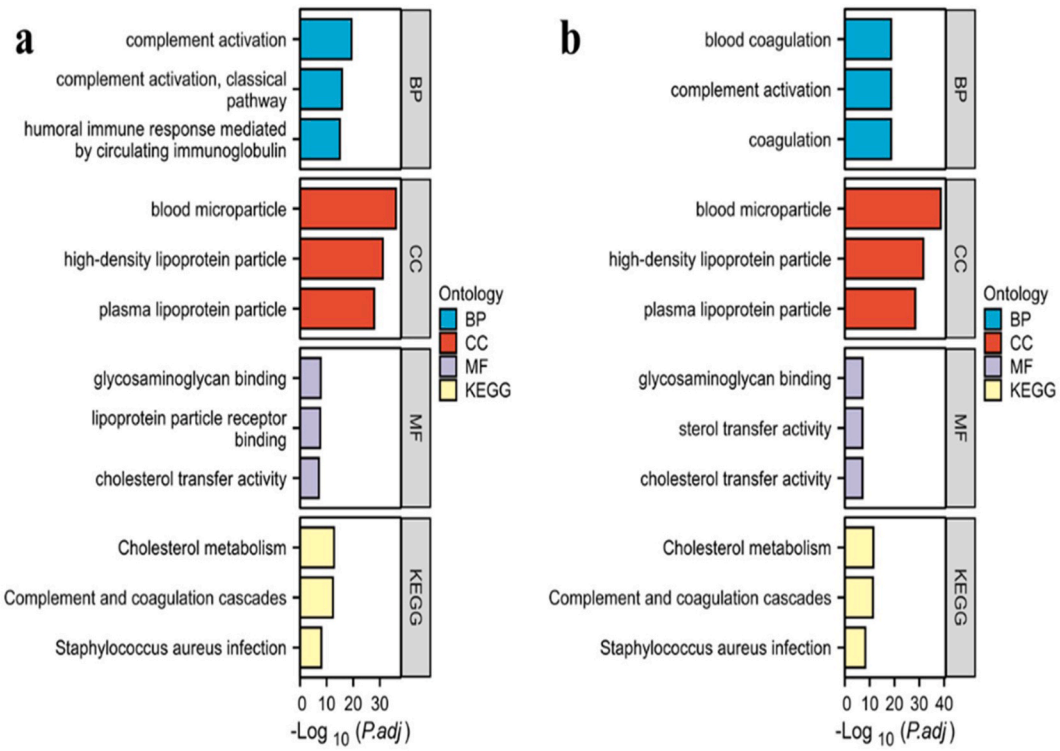


**Fig. 2.** GO and KEGG enrichment analysis. This figure showed biological process, cellular component, molecular function and key pathway in RH group and HTN group. A: the GO and KEGG enrichment analysis of HTN; B: the GO and KEGG enrichment analysis of RH. GO: Gene Ontology; KE**GG**: Kyoto Encyclopedia of Genes and Genomes. HTN: hypertension; RH: resistant hypertension.

*3.1.3.2. Results of up-regulated protein enrichment analysis.* We conducted GO enrichment analysis of the up-regulated proteins, and the results indicated that these proteins were primarily enriched in haemostasis, coagulation, wound healing, and other biological processes. They were involved in cellular components such as cellular lipid metabolism, cell-cell interactions, and cell adhesion, and were associated with molecular functions such as intercellular interactions, protein-lipid interactions, and metabolic activities (Fig. 4). Further KEGG enrichment analysis revealed that the proteomic enrichment of up-regulated proteins was mainly concentrated in pathways such as cell development and differentiation, and infection (Fig. 5).

*3.1.3.3. Differential protein screening.* The up-regulated and down-regulated proteins that were screened underwent ROC curve analysis, and their sensitivities and specificities were calculated. Proteins with an AUC >0.85 and sensitivity and specificity greater than 85 % were defined as differential proteins. A total of 11 differential proteins were identified (Table 2).

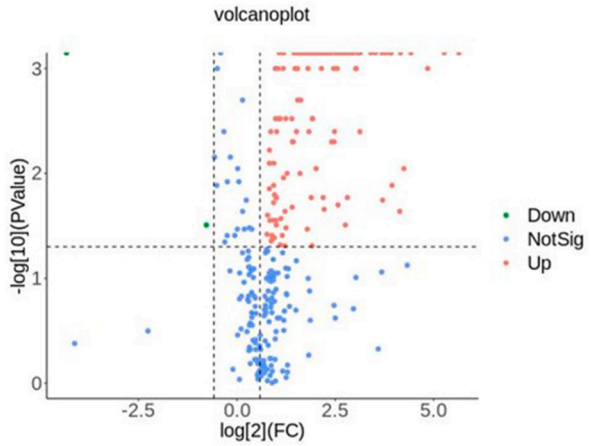


**Fig. 3.** Volcano plot. This figure showed that the two groups revealed 142 up-regulated proteins and 1 down-regulated protein, indicating significant differences in protein expression levels.

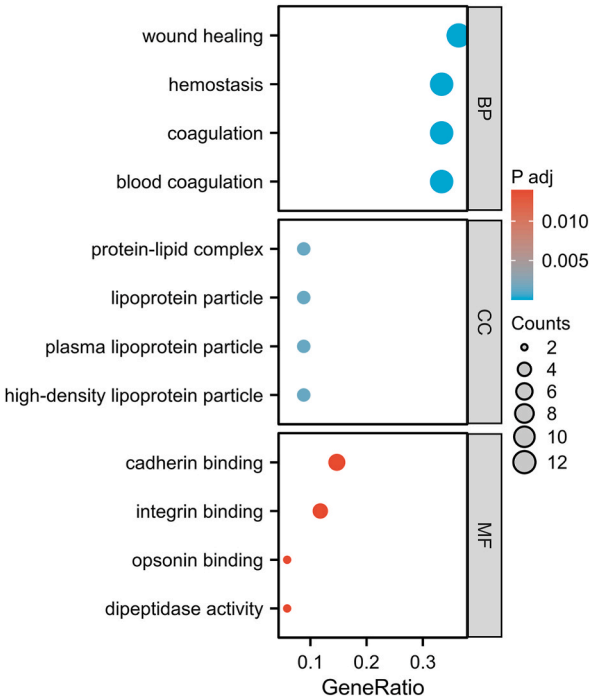


**Fig. 4.** GO enrichment analysis. This figure showed the difference between RH group and HTN group in biological process, cellular component, molecular function. GO: Gene Ontology.

*3.1.3.4. Correlation analysis.* Correlation analysis between the identified differential proteins and patients' baseline data, tests, and examination results revealed significant associations. HV315, ATPB, ENOA, SAA1, APOC4, PLTP, PI16, and TYB4 proteins exhibited correlations with cardiac structural alterations. SAA1, PLTP, PI16, TYB4, and MMRN1 proteins were linked to liver function, while HV351, PLTP, PI16, TYB4, and MMRN1 proteins were correlated with renal function. Moreover, HV315 and PLTP showed correlations with coagulation function, ATPB was associated with sodium ion metabolism, and PLTP, EF1A1, PGBM, and MMRN1 proteins demonstrated correlations with lipid metabolism (Table 3).

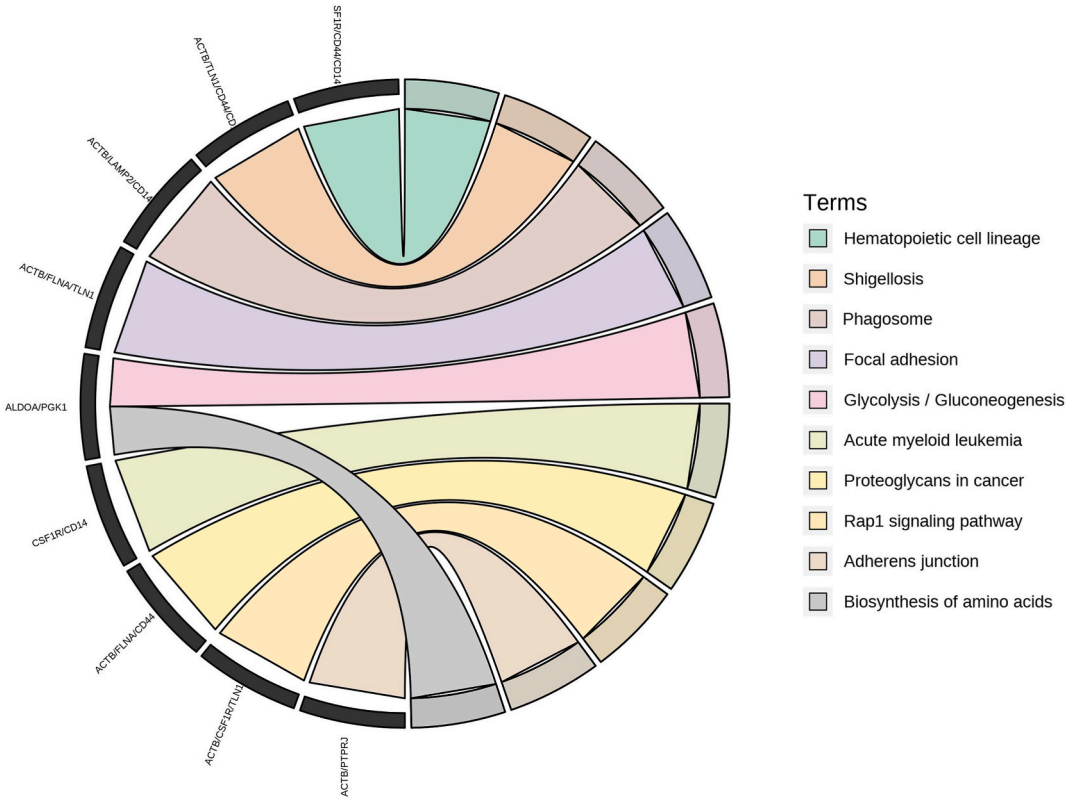


**Fig. 5.** KEGG enrichment analysis. This figure showed the difference between RH group and HTN group in key pathway. KEGG: Kyoto Encyclopedia of Genes and Genomes.

**Table 2**
Differential protein screening.

| protein | AUC | sensitivity | specificity | Youden index |
|---|---|---|---|---|
| PI16 | 0.92 | 85.7 % | 86.7 % | 0.72 |
| SAA1 | 0.94 | 90.0 % | 90.0 % | 0.80 |
| APOC4 | 0.94 | 90.0 % | 90.0 % | 0.80 |
| EF1A1 | 0.97 | 96.0 % | 92.3 % | 0.88 |
| MMRN1 | 0.95 | 89.3 % | 93.3 % | 0.83 |
| TYB4 | 0.94 | 90.0 % | 99.0 % | 0.90 |
| PLTP | 0.92 | 92.0 % | 99.0 % | 0.92 |
| ATPB | 0.96 | 92.9 % | 99.0 % | 0.93 |
| HV315 | 0.99 | 93.3 % | 99.0 % | 0.93 |
| ENOA | 0.99 | 96.0 % | 99.0 % | 0.96 |
| PGBM | 0.97 | 96.4 % | 99.0 % | 0.96 |

AUC: area under the curve.

## 4. Results of the clinical data section

### *4.1. Least absolute selection and shrinkage operator (Lasso)*

Retrospective collection and analysis of clinical data from patients with RH and HTN were conducted. The study encompassed a total of 1322 patients, including 1088 EH patients and 224 RH patients. A comprehensive lasso regression equation incorporated 44 indicators comprising baseline information and laboratory test results. Through cross-validation methods, the λ value yielding the smallest mean square error and the λ value for the simplest model were determined. The optimal number of independent variables was identified as 4, and these 4 indicators were selected for subsequent multifactorial analysis (Fig. 6).

**Table 3**
Correlation analysis.

| protein | | Correlation coefficient | P value | protein | | Correlation coefficient | P value |
|---|---|---|---|---|---|---|---|
| HV315 | SBP | 0.35 | 0.01 | PI16 | EGFR | −0.33 | 0.02 |
| | Cys-C | 0.43 | 0.01 | | Scr | 0.39 | 0.01 |
| | D-D | 0.32 | 0.03 | | UA | 0.39 | 0.01 |
| | LVPW | 0.35 | 0.02 | | RV | 0.32 | 0.03 |
| ATPB | $Na^+$ | −0.36 | 0.01 | TYB4 | TBIL | −.0.33 | 0.02 |
| | IVS | 0.44 | 0.01 | | IBIL | −.0.41 | 0.01 |
| | LVPW | 0.36* | 0.02 | | Cys-C | 0.34 | 0.02 |
| ENOA | RA | −0.33 | 0.03 | | IVS | 0.41 | 0.01 |
| SAA1 | IBIL | −0.35 | 0.01 | | LVPW | 0.33* | 0.03 |
| | IVS | 0.39 | 0.01 | EF1A1 | SBP | 0.42 | 0.01 |
| | LVPW | .0.30 | 0.04 | | LDL | 0.32 | 0.02 |
| APOC4 | IVS | 0.34 | 0.02 | | TC | 0.41 | 0.01 |
| PLTP | TBIL | −0.42 | 0.01 | PGBM | DBP | −0.31 | 0.03 |
| | IBIL | −0.48 | 0.01 | | LDL | −0.28 | 0.04 |
| | TG | −0.30 | 0.03 | | TC | −0.42 | 0.01 |
| | Cys-C | 0.36 | 0.01 | MMRN1 | IBIL | −0.31 | 0.03 |
| | FBG | 0.48 | 0.01 | | Cys-C | 0.33 | 0.02 |
| | D-D | 0.41 | 0.01 | | TG | −0.34 | 0.02 |
| | LVEF | −0.38 | 0.01 | | TC | −.0.37 | 0.01 |
| | IVS | 0.35 | 0.02 | | Scr | 0.30 | 0.04 |
| PI16 | GGT | 0.31 | 0.03 | | | | |

SBP: Systolic Blood Pressure; TBIL: Total Bilirubin; IBIL: Indirect Bilirubin; GGT: Gamma-Glutamyl Transferase; TG: Triglycerides; TC: Total Cholesterol; LDL: Low-Density Lipoprotein; FBG: Fibrinogen; D-D: D-Dimer; EGFR: Estimated Glomerular Filtration Rate; Cys-C: Cystatin C; Scr: Serum Creatinine; UA: Uric Acid; Na: Sodium; LVEF: Left Ventricular Ejection Fraction; IVS: Interventricular Septum; LVPW: Left Ventricular Posterior Wall; RA: Right Atrium; RV: Right Ventricle.

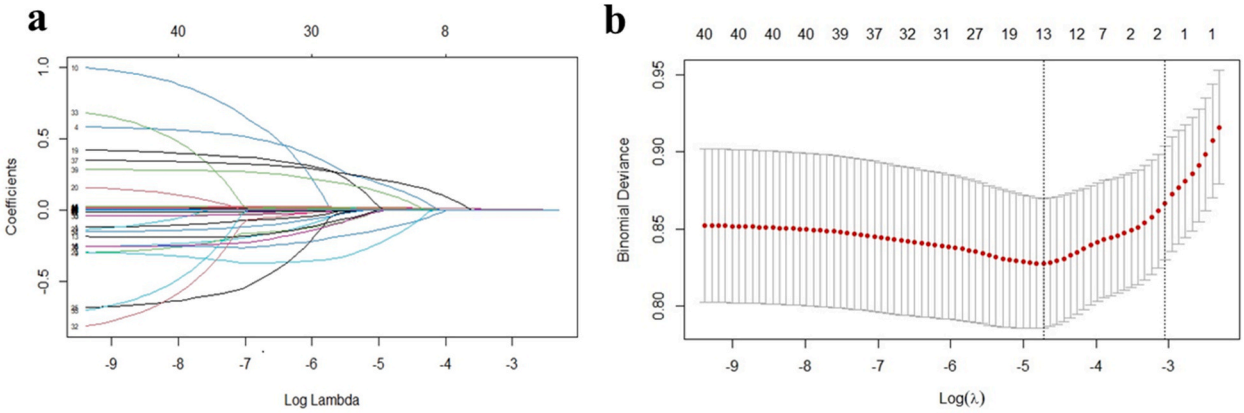


**Fig. 6.** Lasso regression. This figure showed that the optimal number of independent variables was identified as 4, and these 4 indicators were selected for subsequent multifactorial analysis. A represents the result of lasso regression. B represents the result of cross-validation. LASSO, least absolute shrinkage and selection operator.

### *4.2. Multi-factor analysis*

Risk factors contributing to RH were analyzed using RH occurrence as the dependent variable and the risk factors identified in the lasso regression analysis, along with protective factors, as independent variables. SBP, total testosterone (TT), fasting blood glucose

**Table 4**
Multivariate analysis.

| | B | P-value | Exp(B) | 95%CI |
|---|---|---|---|---|
| constant | −6.08 | 0.00 | 0.01 | |
| SBP | 0.02 | 0.00 | 1.02 | 1.01–1.02 |
| TT | 0.02 | 0.00 | 1.02 | 1.01–1.03 |
| FBG | 0.40 | 0.00 | 1.50 | 1.19–1.87 |
| AST | 0.02 | 0.01 | 1.02 | 1.01–1.03 |

SBP: Systolic Blood Pressure; TT: Thrombin Time; FBG: Fibrinogen; AST: Aspartate Aminotransferase.

(FBG), and aspartate transaminase (AST) were identified as significant risk factors for RH (Table 4).

### 4.3. Column line diagram and validation

The multifactorial logistic analysis produced a model equation: Y = (−6.08) + 0.02 (systolic blood pressure) + 0.02 (TT) + 0.40 (FBG) + 0.02 (AST). To visually represent the logistic model, a column-line plot was generated, incorporating the four selected risk factors. AST emerged as the most influential predictor with a maximum score of 100, followed by TT with a maximum score of 98, SBP with a maximum score of 62, and AST with a maximum score of 56 (Fig. 7A). The model-predicted probabilities were plotted on a ROC curve, yielding an area under the curve of 0.65 (95 % CI: 0.60–0.69), a sensitivity of 41.1 %, a specificity of 81.6 %, and a maximum value of 0.227 for the Youden index (Fig. 7B). A calibration curve illustrated excellent calibration with an average error of 0.02 (Fig. 7C). The clinical utility of the model was further assessed through the construction of decision curves, demonstrating the model's net benefit in RH prediction across various probability thresholds (17 %–54 %). This underscores the model's reliability and utility in practical clinical applications (Fig. 7D).

## 5. Discussion

In this study, we observed significant variations in protein expression between individuals with RH and those with HTN due to other causes. Patients with RH showed a unique expression pattern of 29 proteins, in contrast to the 59 proteins uniquely expressed in HTN patients. The proteins in RH patients were predominantly involved in lipid metabolism, protein interaction networks, oxidative stress responses, and metabolic regulation mechanisms. On the other hand, HTN patients' proteins were associated with protein breakdown processes, lipid metabolism, cell adhesion, and oxidative stress.

These differences in protein functionality reflect the unique paths of disease progression and the extent of biological impact for each condition. They may also shed light on the underlying characteristics of different HTN subtypes and the factors that complicate their treatment. To delve deeper into the role of these proteins, we utilized volcano plots to identify 142 proteins that were upregulated and a single protein that was downregulated. Further functional and pathway analyses of the upregulated proteins revealed key biological processes and signaling pathways associated with RH, including hemostasis, coagulation, wound healing, cell adhesion, and immune responses. Additionally, the pathways involved in cell development, differentiation, response to infection, immunity, and metabolism were highlighted.

These discoveries highlight the complexity of RH, which is not just a matter of blood pressure regulation but also involves disruptions across a spectrum of biological processes. Utilizing diagnostic curves, we identified proteins with an Area Under the Curve (AUC) greater than 0.85, along with sensitivity and specificity rates above 85 %, as significant markers of differential expression. Integrating the analysis of these proteins with clinical patient data revealed a strong association between these proteins and various organ functions, including liver and kidney function, coagulation capabilities, and the structure of the heart.

In the study of RH, a discernible variation in the expression levels of proteins ATPB and ENOA was detected, highlighting their efficacy in distinguishing RH from hypertensive patients with better blood pressure (BP) control. ATPB, predominantly found in the inner mitochondrial membrane [13], plays a pivotal role in catalyzing ATP synthesis, promoting oxidative phosphorylation [14], and is implicated in NFκB activation and oxidative stress pathways [15]. Given the high ATP demands of the heart in hypertensive patients to maintain cardiac muscle function against increased load, ATPB production surges. Conversely, ENOA is a metabolic enzyme involved in pyruvate synthesis, expressed on the cell surface, promotes cancer invasion and is subject to specific post-translational modifications [16], which are involved in glycolysis, apoptosis, and cellular stress, as well as in the regulation of vascular tone. In patients with HTN, abnormalities in glycolipid metabolism and cellular stress are usually associated with HTN, leading to elevated ENOA. In patients with HTN, abnormalities in glucose and lipid metabolism and cellular stress are often associated with HTN, leading to increased levels of ENOA; at the same time, HTN also causes increased vascular tone, and ENOA is expressed in vascular endothelial cells. In addition, HTN can cause an increase in vascular tone, and ENOA can be expressed in vascular endothelial cells to regulate vascular tone. Therefore, ENOA can be reactively increased when blood pressure is elevated. The ROC curve analysis demonstrated high diagnostic potential for ATPB with an AUC of 0.96, sensitivity of 92.9 %, and specificity of 99 %, and for ENOA with an AUC of 0.99, sensitivity of 96 %, and specificity of 99 %. This advocates for the consideration of ATPB and ENOA as viable biomarkers for RH identification.

APOC4 and PLTP were found to be differentially expressed in RH patients, with APOC4 located in HDL and VLDL particles and regulating lipid metabolism and lipoprotein assembly [17,18]. Its inhibition of lipoprotein lipase activity is notable in lipid metabolism disorders common in hypertension. APOC4's association with insulin resistance is also significant, as it can interfere with insulin signaling pathways. APOC4 may also affect cardiovascular health because of its association with lipid metabolism and atherosclerosis. Cardiovascular health problems are common in the course of HTN, so APOC4 may play a role in the maintenance of cardiovascular health. PLTP, involved in lipid and cholesterol transport as well as immune regulation, is linked to hyperlipidemia and atherosclerosis [19–21]. The protein's role in lipid molecule transfer, especially in conditions like hyperlipidemia and hypertension, is crucial for lipid distribution regulation in serum. The ROC curve analysis indicated APOC4 with an AUC of 0.94, sensitivity of 90 %, and specificity of 90 %, and PLTP with an AUC of 0.92, sensitivity of 92 %, and specificity of 99 %, suggesting their potential as RH biomarkers.

Serum amyloid A-1 protein (SAA1), synthesized mainly by the liver and elevated during inflammation and tissue injury [22], is linked to cytokine secretion and associated with inflammatory [23] and infectious diseases [24,25]. Its role in cholesterol metabolism and atherosclerosis development, alongside its potential involvement in HTN-related inflammation, positions SAA1 as a candidate biomarker for RH, with an AUC of 0.94, sensitivity of 90 %, and specificity of 90 % in ROC curve analysis.

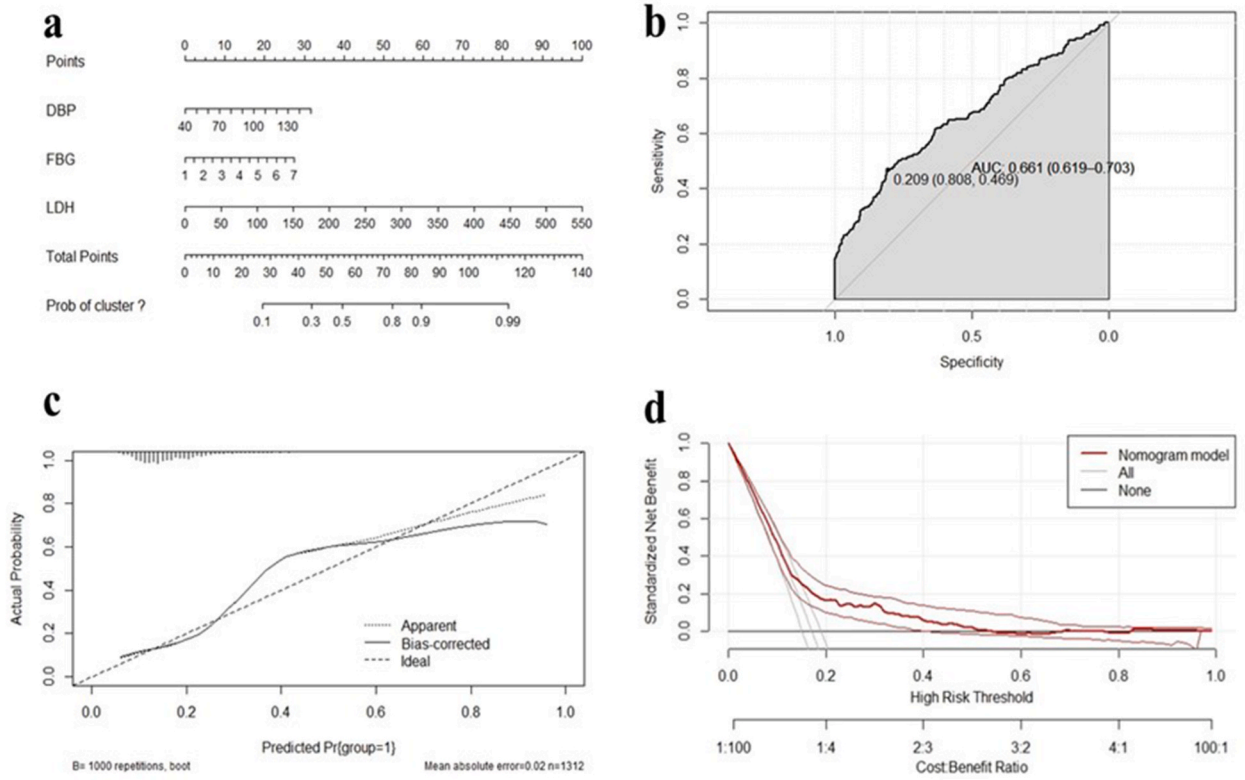


**Fig. 7.** Nomogram plot and validation. A: Nomogram plot, showed that AST emerged as the most influential predictor with a maximum score of 100, followed by TT with a maximum score of 98, SBP with a maximum score of 62, and AST with a maximum score of 56; B: ROC curve, the AUC of the predictive model was 0.65 (95 % CI: 0.60–0.69) with sensitivity and specificity of 41.1 % and 81.6 %; C: Calibration plot, the predictive models had good calibration with a mean error of 0.02; D: DCA curve, the DCA curve showed that using the model to predict the development of RH produced more benefit when the probability of RH between 0.17 and 0.54. ROC: receiver operating characteristic; AUC: area under curve; DCA: decision analysis curve.

Differential expression of proteins PI16, EF1A1, and TYB4 was observed in RH patients. PI16, with anti-inflammatory properties, may counteract HTN-related inflammation. Widely expressed across an array of human tissues, the cysteine-rich protein PI16 is predicted to function as a peptidase inhibitor [26]. It is instrumental in the negative regulation of cell proliferation, notably within the developmental stages of cardiomyocytes [27]. PI16 has been shown to have anti-inflammatory effects. Since chronic inflammation plays a role in the development of HTN, the development of HTN may further lead to an increased production of PI16 to exert its anti-inflammatory effects and reduce the inflammatory response in HTN patients. EF1A1, mainly residing within the cell, assumes a critical role in protein synthesis, participating in peptide chain elongation, binding and release of tRNAs, and regulating cytoskeleton [28] and cell movement [29]. The main biological function of EF1A1 is to catalyze the joining of amino acids and participate in the synthesis of new protein chains. In patients with HTN, cells often need to synthesize new proteins to meet metabolic demands, so the activity and expression level of EF1A1 may increase with HTN to regulate protein synthesis. TYB4 primarily resides within cells, influencing cell proliferation, migration, and differentiation, and plays a vital role in cytoskeleton organization [30]. TYB4's anti-inflammatory properties are instrumental in reducing inflammation, a critical factor in the progression of hypertension (HTN). Chronic inflammation is closely tied to the advancement of the disease and the emergence of complications. The escalation of blood pressure during HTN may lead to an increase in TYB4 levels, potentially serving as a protective mechanism against inflammation. TYB4's influence extends beyond inflammation; it also plays a crucial role in tissue repair and healing processes. In the context of HTN, where damage and subsequent repair of cardiac and vascular tissues are common, TYB4's involvement is likely essential for maintaining tissue health and integrity. Studies have demonstrated the close correlation of the three proteins with cancer [31–33]. The association of these proteins with cancer has been noted, and their diagnostic potential, with AUCs ranging from 0.92 to 0.97 and high sensitivity and specificity, recommends their consideration as RH biomarkers.

MMRN1 is mainly found in platelets, endothelial cells, and extracellular matrix, participating in the coagulation process and regulates platelet function. In addition, it has been found that MMRN1 is closely related to the development of cancer, and can be used as a biomarker for a variety of cancers [34,35]. The occurrence and development of HTN are often accompanied by abnormalities in blood coagulation and platelet activity, and the presence of MMRN1 may play a role in these processes. The ROC curve analysis revealed that the AUC of differential protein MMRN1 was 0.95, sensitivity was 89.3 %, and specificity was 93.3 %. Hence, we

recommend considering MMRN1 as a promising biomarker for distinguishing RH from HTN.

Clinical data from a cohort of 1088 patients with HTN and 224 with RH were rigorously analyzed to identify risk factors for RH, including systolic blood pressure, total triglycerides, fasting blood glucose, and aspartate aminotransferase. A predictive model based on these factors showed modest diagnostic efficacy, underlining the need for further research into proteomic differences and the influence of genetic and environmental factors in RH. The study's limitations, including sample size and lack of experimental validation, are acknowledged, with a call for future research to explore these areas more comprehensively.

## 6. Conclusion

In summary, this study employed proteomics technology to elucidate the protein expression profiles characteristic of patients with resistant hypertension, distinguishing them from those with standard hypertension. Our investigation pinpointed 11 proteins—PI16, SAA1, APOC4, EF1A1, MMRN1, TYB4, PLTP, ATPB, HV315, ENOA, and PGBM—manifesting significant differential expression in patients with resistant hypertension. These proteins hold promise as serological biomarkers for resistant hypertension, demonstrating high diagnostic efficacy.

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## Ethical approval

Human subjects/informed consent statement approval for the study was obtained from the Ethics Committee of Jinan Central Hospital, under Ethics No. 2021-213-01. Informed consent was obtained from all individual participants included in the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5).

## Data availability

The data presented in this study are available on request from the corresponding author.

## CRediT authorship contribution statement

**Xiaoqian Yu:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jianmin Du:** Formal analysis, Data curation. **Wenyu Zhang:** Methodology, Investigation, Formal analysis, Data curation. **Xinghai Zhang:** Data curation. **Hengli Zhao:** Conceptualization. **Qing Wen:** Writing – review & editing, Conceptualization. **Rui Xu:** Writing – review & editing, Conceptualization.

## Declaration of AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used chat gpt 3.5 in order to polish. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

## Declaration of competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article. The authors declare no competing interests.

## References

[1] A. Cai, C. Zheng, J. Qiu, G.C. Fonarow, G.Y.H. Lip, Y. Feng, et al., Prevalence of heart failure stages in the general population and implications for heart failure prevention: reports from the China Hypertension Survey 2012-15, Eur J Prev Cardiol 30 (2023) 1391–1400.
[2] M. Siddiqui, D.A. Calhoun, Refractory versus resistant hypertension, Curr. Opin. Nephrol. Hypertens. 26 (2017) 14–19.
[3] S. Hiremath, R. Sapir-Pichhadze, M. Nakhla, J.Y. Gabor, N.A. Khan, L.M. Kuyper, et al., Hypertension Canada's 2020 evidence review and guidelines for the management of resistant hypertension, Can. J. Cardiol. 36 (2020) 625–634.
[4] E. Pimenta, D.A. Calhoun, Resistant hypertension: incidence, prevalence, and prognosis, Circulation 125 (2012) 1594–1596.
[5] C. Tsioufis, A. Kasiakogias, A. Kordalis, K. Dimitriadis, C. Thomopoulos, D. Tsiachris, et al., Dynamic resistant hypertension patterns as predictors of cardiovascular morbidity: a 4-year prospective study, J. Hypertens. 32 (2014) 415–422.
[6] A.Y. Hwang, E. Dietrich, C.J. Pepine, S.M. Smith, Resistant hypertension: mechanisms and treatment, Curr. Hypertens. Rep. 19 (2017) 56.
[7] C. Delles, E. Carrick, D. Graham, S.A. Nicklin, Utilizing proteomics to understand and define hypertension: where are we and where do we go? Expert Rev. Proteomics 15 (2018) 581–592.
[8] T.T. Duarte, C.T. Spencer, Personalized proteomics: the future of precision medicine, Proteomes 4 (2016) 29.
[9] P.R. Gajjala, V. Jankowski, G. Heinze, G. Bilo, A. Zanchetti, H. Noels, et al., Proteomic-biostatistic integrated approach for finding the underlying molecular determinants of hypertension in human plasma, Hypertension 70 (2017) 412–419.

[10] Z. Liu, Research of the Differential Expression of Proteins of Left Ventricular Myocardium in Hypertensive Heart Disease, 2016.
[11] S. Liu, Y. Zhang, S. Chen, J. lin, M. Lin, Q. Yang, et al., Proteomic analysis of cerebrospinal fluid proteins in patients with HTN intracerebral hemorrhage before and after surgery, Journal of Chinese Gerontology 33 (2013) 2655–2656.
[12] T. Liu, Study on specific serum proteins of HTN-induced heart and kidney damage, in: High-Density Lipoprotein Cholesterol Levels Correlate with Overall Mortality in Patients with Cerebral Thrombosis [D], Peking Union Medical College, 2017.
[13] M. Martin-Lorenzo, P.J. Martinez, M. Baldan-Martin, J.A. Lopez, P. Minguez, A. Santiago-Hernandez, et al., Urine haptoglobin and haptoglobin-related protein predict response to spironolactone in patients with resistant hypertension, Hypertension 73 (2019) 794–802.
[14] W. Li, Y. Li, G. Li, Z. Zhou, X. Chang, Y. Xia, et al., Ectopic expression of the ATP synthase β subunit on the membrane of PC-3M cells supports its potential role in prostate cancer metastasis, Int. J. Oncol. 50 (2017) 1312–1320.
[15] K. Anjum, H. Bi, W. Chai, X.Y. Lian, Z. Zhang, Antiglioma pseurotin A from marine bacillus sp. FS8D regulating tumour metabolic enzymes, Nat. Prod. Res. 32 (2018) 1353–1356.
[16] L. Miao, Z. Zhuo, J. Tang, X. Huang, J. Liu, H.Y. Wang, et al., FABP4 deactivates NF-κB-IL1α pathway by ubiquitinating ATPB in tumor-associated macrophages and promotes neuroblastoma progression, Clin. Transl. Med. 11 (2021) e395.
[17] M. Capello, S. Ferri-Borgogno, P. Cappello, F. Novelli, α-Enolase: a promising therapeutic and diagnostic tumor target, FEBS J. 278 (2011) 1064–1074.
[18] L. Kotite, L.H. Zhang, Z. Yu, A.L. Burlingame, R.J. Havel, Human apoC-IV: isolation, characterization, and immunochemical quantification in plasma and plasma lipoproteins, J. Lipid Res. 44 (2003) 1387–1394.
[19] C.M. Allan, J.M. Taylor, Expression of a novel human apolipoprotein (apoC-IV) causes hypertriglyceridemia in transgenic mice, J. Lipid Res. 37 (1996) 1510–1518.
[20] T. Gautier, V. Deckert, M. Nguyen, C. Desrumaux, D. Masson, L. Lagrost, New therapeutic horizons for plasma phospholipid transfer protein (PLTP): targeting endotoxemia, infection and sepsis, Pharmacol. Ther. 236 (2022) 108105.
[21] X.C. Jiang, Y. Yu, The role of phospholipid transfer protein in the development of atherosclerosis, Curr. Atherosclerosis Rep. 23 (2021) 9.
[22] J.J. Albers, S. Vuletic, M.C. Cheung, Role of plasma phospholipid transfer protein in lipid and lipoprotein metabolism, Biochim. Biophys. Acta 1821 (2012) 345–357.
[23] C.M. Uhlar, A.S. Whitehead, Serum amyloid A, the major vertebrate acute-phase reactant, Eur. J. Biochem. 265 (1999) 501–523.
[24] H. Patel, R. Fellowes, S. Coade, P. Woo, Human serum amyloid A has cytokine-like properties, Scand. J. Immunol. 48 (1998) 410–418.
[25] N. Cheng, Y. Liang, X. Du, R.D. Ye, Serum amyloid A promotes LPS clearance and suppresses LPS-induced inflammation and tissue injury, EMBO Rep. 19 (2018) e45517.
[26] B. Jiang, D. Wang, Y. Hu, W. Li, F. Liu, X. Zhu, et al., Serum amyloid A1 exacerbates hepatic steatosis via TLR4-mediated NF-κB signaling pathway, Mol. Metabol. 59 (2022) 101462.
[27] G.M. Gibbs, K. Roelants, M.K. O'Bryan, The CAP superfamily: cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins–roles in reproduction, cancer, and immune defense, Endocr. Rev. 29 (2008) 865–897.
[28] M. Regn, B. Laggerbauer, C. Jentzsch, D. Ramanujam, A. Ahles, S. Sichler, et al., Peptidase inhibitor 16 is a membrane-tethered regulator of chemerin processing in the myocardium, J. Mol. Cell. Cardiol. 99 (2016) 57–64.
[29] J. Zhang, H. Guo, Z. Mi, C. Gao, S. Bhattacharya, J. Li, et al., EF1A1-actin interactions alter mRNA stability to determine differential osteopontin expression in HepG2 and Hep3B cells, Exp. Cell Res. 315 (2009) 304–312.
[30] D. Zhong, J. Zhang, S. Yang, U.J. Soh, J.P. Buschdorf, Y.T. Zhou, et al., The SAM domain of the RhoGAP DLC1 binds EF1A1 to regulate cell migration, J. Cell Sci. 122 (2009) 414–424.
[31] R.E. Zoubek, E. Hannappel, Subcellular distribution of thymosin beta4, Ann. N. Y. Acad. Sci. 1112 (2007) 442–450.
[32] X. Kuang, Z. Zhang, D. Li, W. Bao, J. Pan, P. Zhou, et al., Peptidase inhibitor (PI16) impairs bladder cancer metastasis by inhibiting NF-κB activation via disrupting multiple-site ubiquitination of NEMO, Cell. Mol. Biol. Lett. 28 (2023) 62.
[33] L. Yi, S. He, Z. Cheng, X. Chen, X. Ren, Y. Bai, DNAJA1 stabilizes EF1A1 to promote cell proliferation and metastasis of liver cancer mediated by miR-205-5p, JAMA Oncol. 2022 (2022) 2292481.
[34] M. Dettin, F. Ghezzo, M.T. Conconi, L. Urbani, G. D'Auria, L. Falcigno, et al., In vitro and in vivo pro-angiogenic effects of thymosin-β4-derived peptides, Cell. Immunol. 271 (2011) 299–307.
[35] A. Colombatti, P. Spessotto, R. Doliana, M. Mongiat, G.M. Bressan, G. Esposito, The EMILIN/Multimerin family, Front. Immunol. 2 (2012) 93.