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Unveiling Key Pathways and Potential Biomarkers for High-Altitude Hypertension: A Pilot Multi-Omics Study[1]

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[1] Abbreviations: cardiovascular (CV); Genome-Wide Association Studies (GWAS); oviduct glycoprotein 1 (OVGP1); myosin heavy chain IIA (MYH9, nonmuscle myosin heavy chain IIA); healthy controls (HC); data-independent acquisition (DIA); data-dependent acquisition (DDA); liquid-liquid mass spectrometry (LC-MS/MS); Parallel reaction monitoring (PRM); Orthogonal Partial Least Squares -Discriminant Analysis (OPLS-DA); Receiver operating characteristic (ROC); random forest (RF); partial least squares discriminant analysis (PLS-DA); immunoglobulin heavy variable 3-15 (IGHV3-15); Uteroglobin (SCGB1A1); immunoglobulin lambda-like polypeptide 1 (IGLL1); glucoside xylosyltransferase 2 (GXYLT2); superoxide dismutase [Cu-Zn] (SOD1); prelamin-A/C (LMNA); cathepsin G (CTSG); creatine kinase B-type (CKB); ACTG1 (actin, gamma 1); APOB (apolipoprotein B); MPO (myeloperoxidase); ACTN1 (alpha-actinin-1); C8A (component C8 alpha chain); CRISP3 (cysteine-rich secretory protein 3); CTSG (cathepsin G); YWHAZ (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta); APOF (apolipoprotein F); AZGP1 (alpha-2-glycoprotein 1, zinc-binding); IGLV5-39 (immunoglobulin lambda variable 5-39); H2AC8 (histone H2A type 1-C/E/F/G/I); LMNA (lamin A/C); PLTP (phospholipid transfer protein); SERPINA11 (serpin family A member 11); CKB (creatine kinase B 1, zinc-binding); ECM1 (extracellular matrix protein 1); VNN1 (vanin 1); LTA4H (leukotriene A4 hydrolase); and GXYLT2 (glucoside xylosyltransferase 2)

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Abstract

OBJECTIVE: High altitude has a considerable impact on the pathophysiology of the human cardiovascular system and disease occurrence. We aim to use an integrated approach of metabolomics and proteomics to reveal key pathways and biomarkers of hypertension at high altitude.
METHODS: Thirty Tibetan patients with hypertension and 30 healthy individuals residing on the Tibetan Plateau at a very high altitude (> 4500m) were included in the study. Metabolomic analysis was conducted using Vanquish ultra-high performance liquid chromatography, while proteomic analysis utilized the timsTOF Pro2 mass spectrometer. Correlation analysis revealed key signaling pathways and biomarkers associated with hypertension in Tibetan patients.
RESULTS: The results showed 87 differentially expressed metabolites and 61 differentially expressed proteins in individuals with hypertension at high altitude. The results of metabolomic differential metabolite pathway analysis indicated that Caffeine metabolism had the most significant impact. Specific metabolites like PI(16:0/16:0), Caffeine, and Plastoquinone 3 were found to be significantly up-regulated in hypertensive patients. The combination of five metabolites achieved an area under the curve (AUC) of 0.871 for hypertension prediction. Proteomic analysis revealed that the identified differential proteins primarily functioned in signaling receptor binding. It was confirmed that Creatine kinase B (CKB) and Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) could serve as a protein biomarker combination for hypertension in plateau regions, showing an AUC of 0.764 (0.585–0.944). Upon conducting an integrated analysis of metabolomics and proteomics, the combined AUC improved to 0.982 (0.949–1.000).
CONCLUSION: A comprehensive analysis utilizing metabolomics and proteomics revealed that alterations in signal transduction-related pathways and lipid metabolism pathways were implicated in hypertension among plateau populations. Additionally, YWHAZ was observed as a potential biomarker for this condition.
Key words: high altitude; hypoxia; hypertension; metabolomics; proteomics;

1. Introduction

Hypertension, a major risk factor for cardiovascular disease, is responsible for 10.5 million of the 18 million cardiovascular (CV) deaths that occur

annually, accounting for 12% of all global deaths [1]. By 2025, more than 1.5 billion individuals worldwide are projected to be affected by hypertension. Epidemiological and genomic studies have highlighted the crucial link between hypertension incidence and environmental factors[2]. Plateau environments, such as those inducing hypoxia, trigger various physiological and pathological changes, like sympathetic nervous system activation, contributing to a high prevalence of hypertension in these areas [3–5]. The prevalence of hypertension tends to rise with increasing altitude[6]. Despite this, the precise mechanism underlying the elevated prevalence of hypertension in plateau regions remains unclear.

Tibetans, known for their adaptability to plateau conditions, exhibit unique physiological traits, such as higher capillary density, enhanced oxygen transport, and elevated blood oxygen saturation, compared to individuals in the Andean plateau. Genetic analyses of the Tibetan population living on plateaus have identified genes associated with high-altitude adaptation and superior adaptability [7–15]. This group serves as an ideal cohort for investigating the pathogenesis of plateau-related diseases, including hypertension. Genome-Wide Association Studies (GWAS) have identified over 1,000 blood pressure-related loci, leading to the discovery of 436 candidate genes linked to blood pressure regulation [16]. Throughout the development of hypertension, critical organs and tissues undergo significant functional and structural changes, characterized by variations in the expression of proteins in terms of quantity and type[17].

Studies on hypertension in plain regions identified key proteins and pathways. Nitric Oxide Synthase 3 (NOS3), Chitinase-3-like protein 1 (CHI3L1), and 34 others, including protective Transforming Growth Factor Beta Receptor 3 (TGFBR3) and Prostaglandin D2 Synthase (PTGDS), are linked to inflammation, vascular function, hormonal regulation (e.g., aldosterone pathway), immunity, and lipid metabolism (e.g., Notch signaling). Elevated Oviduct Glycoprotein 1 (OVGP1) levels correlate with increased blood pressure, vasoconstriction, diastolic dysfunction, arterial remodeling, and vascular oxidative stress. These findings highlight inflammatory, vascular, and metabolic pathways in hypertension, emphasizing gene-environment interactions and potential biomarkers[18–20].

Metabolomics and proteomics are commonly used methods to investigate disease pathogenesis. Meanwhile, there is a lack of multi-omics studies on the occurrence of hypertension in high altitudes. This study is the first to employ a multi-omics approach to explore the pathogenesis of hypertension in Tibetans residing on the plateau, uncovering novel pathways and potential biomarkers related to hypertension in high-altitude regions.

2. Methods

The HI-VHA (Health Improvement of the Very high-altitude) project was

initiated on June 24, 2021, with registration number ChiCTR2100047945. From June to August 2021, individuals living above 4,500 meters in the Nagqu region of Tibet Autonomous Region P.R.C were included in the study based on the 2023 diagnostic guidelines from the European Society of Hypertension (ESH)[21]. 30 hypertensive patients with systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 mmHg were selected, along with thirty healthy Tibetan individuals at the same altitude as healthy controls (HC)(Table 1). Exclusion criteria comprised coronary atherosclerotic heart disease, diabetes, chronic obstructive pulmonary disease, heart failure, significant liver and kidney abnormalities, and a smoking history. The research protocol received approval from the Clinical Research Ethics Committee of Tibet Autonomous Region People's Hospital (ME-TBHP-21-028), and all participants provided informed consent in writing (Fig. 1). This study adheres to the Declaration of Helsinki.

Blood samples were collected from patients and healthy subjects using tubes, coagulated at room temperature for 30 minutes, and then centrifuged at 3000×g for 10 minutes to isolate serum. The serum was subsequently stored at -80°C until analysis.

For metabolomics detection, chromatographic separation of target compounds was performed using Vanquish ultra-high performance liquid chromatography and a Waters ACQUITY UPLC BEH Amide liquid chromatography column (2.1 mm × 100 mm, 1.7 μm). The aqueous phase (Phase A) of the liquid chromatography contained 25 mmol/L of ammonium acetate and 25 mmol/L of ammonia water, while the organic phase (Phase B) was acetonitrile. Gradient elution: 0–0.5 min, 95% B; 0.5–7 min, 95%-65% B; 7-8 min, 65% B-40% B; 8-9 min, 40% B; 9-9.1 min, 40%-95% B; 9.1-12 min, 95% B), column temperature 30°C, injector temperature 4°C, flow rate 0.5 mL/min, injection volume 2 μL. Mass spectrometry data were collected using an Orbitrap Exploris 120 mass spectrometer controlled by Xcalibur software (version 4.4, Thermo). Detailed parameters included sheath gas flow rate of 50 Arb, aux gas flow rate of 15 Arb, capillary temperature of 320 ℃, full ms resolution of 60000, MS/MS resolution of 15000, collision energy of 10/30/60 in NCE mode, and spray voltage of 3.8 kV (positive) or -3.4 kV (negative).

For proteomics detection, data-independent acquisition (DIA) mode was utilized to analyze protein enzymatic peptide fragments through liquid mass spectrometry (LC-MS/MS). A spectral library was first constructed in data-dependent acquisition (DDA) mode, followed by DIA mode analysis of each experimental sample using professional library search software for proteome identification and quantification, including information on peptides, proteins, and protein expression changes. The entire system consists of a timsTOF Pro2 mass spectrometer (Bruker Daltonics) connected in series with the UltiMate

3000 system (Thermo Fisher Scientific, MA, USA). The mass spectrometer operates in DDA PASEF mode with specific parameters: 10 PASEF scans per cycle, 100 ms accumulation time, scanning range from 100 to 1700m/z, and ion mobility (1/K0) range set at 0.6-1.6. The number of scanned charges ranges from 0 to 5, with a target value of 10,000 and a dynamic exclusion time of 0.4 min. Isolation window width is set at 2Th for m/z 700.

Peptides were dissolved in a 0.1% formic acid solution, with 200 ng of sample loaded onto an analytical column (25 cm × 75 μm id, IonOpticks). The sample underwent separation over 80 minutes at 5°C, with a total of 2.5 μL loaded and a column flow rate of 300 nL/min. The gradient started at 4% phase B, increasing to 28% in 45 minutes, then to 44% in 10 minutes, reaching 90% in 10 minutes, and maintained for 7 minutes before returning to 4% for equilibrium. The mass spectrometer used diaPASEF mode for DIA data acquisition, scanning from 349-1229 m/z with a 40 Da isolation window width. During the PASEF MSMS scan, collision energy increased linearly with ion mobility from 59eV (1/K0 = 1.6 Vs/cm2) to 20eV (1/K0 = 0.6 Vs/cm2).

DDA Data Retrieval: DDA data were processed using Spectronaut 16 (Biognosys AG) with default parameters. The sequence database was uniprot-homo sapiens (version 2022, 20,610 entries), with Trypsin digestion and a maximum missed cleavage count of 2. Fixed modifications for database search: Carbamidomethylation (C) 57.02. Variable modifications: Oxidation (M) 15.99. False discovery rate (FDR) set to 1% at the parent ion level and 1% at the protein level.

DIA Data Retrieval: DIA data were analyzed using Spectronaut16 (Biognosys AG) with default parameters. Retention time and mass windows were automatically corrected based on iRT peptide software, which also determined optimal window selection. Protein identification criteria: FDR at the parent ion level was set to 1%, and FDR at the protein level was set to 1%. The decoy database was generated using a mutated strategy, similar to randomly shuffling amino acid sequences (minimum 2 amino acids, maximum half the total peptide length). Spectronaut performed automatic correction and applied a local normalization strategy for data normalization. The average peak area of the top 3 peptides with an FDR below 1.0% was used for protein group quantification.

Proteomics validation through parallel reaction monitoring (PRM) is a targeted detection method that utilizes a high-resolution mass spectrometer (Q-Orbitrap/Q-TOF) to reduce background impurity interference, enhancing selectivity and specificity. In each sampling cycle, preset precursor ions are selected for fragmentation, and all fragment ions are collected simultaneously. Peptides totaling approximately 2 μg were separated using a nano-UPLC liquid phase system (EASY-nLC1200) and then analyzed with a mass spectrometer equipped with a nanoliter ion source (Q-Exactive HFX) for data collection. Chromatographic separation utilized a 100 μm ID × 15 cm reversed-phase

column (Reprosil Pur 120 C18-AQ, 1.9 μm, Dr. Maisch). The mobile phase consisted of an acetonitrile-water-formic acid system, with phase A being 0.1% formic acid in a 98% aqueous solution (2% acetonitrile) and phase B being 0.1% formic acid in an 80% acetonitrile solution (20% water). Following equilibration of the chromatographic column with 100% phase A, the sample was loaded onto the column from the autosampler and gradient separated with a flow rate of 300 nL/min over 60 minutes. The gradient profile for mobile phase B was as follows: 5% for 2 min, 5-30% for 42 min, 30-48% for 8 min, 48-95% for 2 min, and 95% for 6 min. Mass spectrometry analysis employed the parallel reaction monitoring (PRM) method in positive ion detection mode, with a quadrupole isolation window of 0.7 m/z, normalized collision energy (NCE) set at 27%, and a secondary scanning resolution of 15k.

3. Results

Table 1: Study participant demographic characteristics[2]

	Hypertension patients	HC	P-value
Gender (F/M)	21/9	21/9	
Age (years)	44.70 ± 10.41	42.73 ± 10.30	0.465
SBP (mmHg)	141.5 ± 20.2	110.0 ± 10.3	0.000*
DBP (mmHg)	101.9 ± 11.1	75.2 ± 6.8	0.000*
Body mass index (kg/m^2)	25.51 ± 5.64	22.14 ± 1.91	0.004*
Altitude (m)	4625.57 ± 110.96	4667.4 ± 136.96	0.199
Hb (g/L)	166.90 ± 15.49	162.07 ± 14.48	0.217
GLU (mmol/L)	4.47 ± 0.47	4.48 ± 0.44	0.933
TG (mmol/L)	1.17 ± 0.60	0.7 ± 0.22	0.000*
CHOL (mmol/L)	4.70 ± 0.86	4.41 ± 0.84	0.190
LDL-C (mmol/L)	2.85 ± 0.72	2.54 ± 0.69	0.092
HDL-C (mmol/L)	1.35 ± 0.27	1.50 ± 0.28	0.041*
HCY (μmol/L)	16.55 ± 6.31	17.07 ± 6.42	0.751
UA (μmol/L)	344.83 ± 76.47	315.10 ± 83.28	0.155

[2] Abbreviations: Systolic blood pressure(SBP); Diastolic blood pressure(DBP); Hemoglobin(Hb); Glucose(GLU); Triglyceride(TG); Cholesterol(CHOL); Low-density lipoprotein(LDL-C); High density lipoprotein(HDL-C); Homocysteine(HCY); Uric acid(UA)

This study utilized a multi-omics approach to identify potential biomarkers in patients with hypertension. Initially, 30 serum samples from each group in the cohort were randomly selected for non-target metabolomics testing, followed by Kyoto Encyclopedia of Genes and Genomes (KEGG)[22] pathway analysis and metabolite biomarker screening of differentially expressed metabolites. Subsequently, proteomics was employed to identify protein biomarkers in the screening samples, with the results being verified. Finally, an integrated analysis of proteomic and metabolomic data from the same biological samples was conducted, revealing potential biomarkers and pathways associated with the development and progression of hypertension.

A total of 1163 metabolites were identified after denoising, normalizing, and standardizing the assay results. The screening of differentially expressed metabolites (DEMs) combined univariate and multivariate statistical analyses. First, univariate analysis was performed using Student's t-test ($P < 0.05$) with false discovery rate (FDR) correction, as implemented by the Benjamini-Hochberg method (adjusted P-value < 0.05). Second, the variable importance in projection (VIP) of metabolites was assessed using an orthogonal partial least squares discriminant analysis (OPLS-DA) model. Finally, metabolites satisfying both VIP > 1.0 and adjusted $P < 0.05$ were defined as differentially expressed metabolites. Based on this criterion, we identified one significantly downregulated differential metabolite: PC(P-18:1(9Z)/16:0). The limited number of significant findings following FDR correction prompted a further exploratory analysis using less stringent criteria (VIP > 1, $P < 0.05$), which revealed a total of 87 differentially expressed metabolites, including both upregulated and downregulated ones. The differential metabolites were subjected to visual analyses such as orthogonal partial least squares-discriminant analysis (OPLS-DA) (Fig. 2A), hierarchical clustering (Fig. 2B), and volcano diagrams (Fig. 2C). KEGG enrichment analysis of the pathways relevant to the differential metabolites (Fig. 2E) was also performed. By analyzing some of the up- and down-regulated metabolites, 2,3-dinor-TXB2, PC [20:1(11Z)/14:1(9Z)], panaquinquecol 1, tricosanoic acid, 3-acetyl-2,7-naphthyridine, PI (16:0/16:0), PC [20:4(5Z,8Z,11Z,14Z)/20:1(11Z)], caffeine (Figure 2I), prostaglandin B1, N-[(4E,8Z)-1,3-dihydroxyoctadeca-4,8-dien-2-yl] hexadecanamide 1-glucoside, 2'-O-methylcajanone, pseudopelletierine, plastoquinone 3, oleamide, adipic acid, PC [P-18:1(9Z)/16:0], PC [18:2(9Z,12Z)/P-18:1(11Z)], PC [20:4(8Z,11Z,14Z,17Z)/P-18:0], and norambreinolide were identified. Of these, PI (16:0/16:0), caffeine, plastoquinone 3, 2'-O-methylcajanone, and pseudopelletierine were the most upregulated, and norambreinolide, tricosanoic acid, ethyl (2E,6Z)-dodecadienoate, cis-quinceoxepane, and PC [P-18:1(9Z)/16:0] were the most downregulated. Receiver operating characteristic (ROC) analyses were performed for the

five most upregulated metabolites, and an area under the curve (AUC) of 0.871 (0.738–1.000) was determined (Fig. 2D).

The differential metabolite pathway and network analysis in serum highlighted pathways like Linoleic acid metabolism, alpha-Linolenic acid metabolism, Caffeine metabolism, and Arachidonic acid metabolism as the most involved after KEGG enrichment analysis(Fig. 2E & 2F). Figure 2G shows the differential metabolite network analysis.

Machine learning was conducted to assess the value of identified metabolites in predicting hypertension. Random forest (RF) and partial least squares discriminant analysis (PLS-DA) algorithms were employed. The top 11 differentially expressed metabolites in RF were presented in Figure 2H. Notably, 2,3-Dinor-TXB2 (a metabolite of fatty acyl eicosane-thromboxane), 2'-O-Methylcajanone, and Plastoquinone 3 were identified as the three most differentially expressed metabolites in patients with hypertension.

Additionally, proteomic results comparing hypertensive and healthy controls were obtained using DIA technology. After preprocessing, 663 detected proteins were analyzed. Following false discovery rate (FDR) correction (FDR < 0.05), only apolipoprotein F (APOF) was identified as a significantly downregulated differentially expressed protein (Fold Change ≥ 1.2 or ≤ 0.83). To further explore potential differentially expressed proteins and reduce the omission of true positives, we identified a total of 61 differentially expressed proteins based on an uncorrected significance threshold (Fold Change ≥ 1.2 or ≤ 0.83 and $P < 0.05$). A clustering of differentially expressed proteins heatmap (Fig. 3A), a volcano map of differentially expressed proteins(Fig. 3B), and clusters of orthologous groups (COGs) analysis of differentially expressed proteins (Fig. 3C). GO enrichment analysis revealed that most differential proteins were associated with processes outside cells, with immune and defense systems being prominent in Biological Process (GO_BP), and signal receptor binding and immunoglobulin receptor binding being significant in Molecular Function (GO_MF). The differentially expressed proteins were also visualized using a volcano plot(Figure 3F). The volcano plot highlighted the five proteins with the most significant down-regulated and up-regulated expressions. The down-regulated proteins included Immunoglobulin heavy variable 3-15 (IGHV3-15), Immunoglobulin heavy variable 1-18 (IGHV1-18), Immunoglobulin heavy variable 4-34 (IGHV4-34), Uteroglobin (SCGB1A1), and Immunoglobulin lambda-like polypeptide 1 (IGLL1). On the other hand, the upregulated proteins were Glucoside xylosyltransferase 2 (GXYLT2), Superoxide dismutase [Cu-Zn] (SOD1), Prelamin-A/C (LMNA), Cathepsin G (CTSG), and Creatine kinase B-type (CKB). A total of 20 important proteins were validated using PRM (mass spectrometry-based targeted protein validation), including ACTG1, APOB, MPO, ACTN1, C8A, CRISP3, CTSG, YWHAZ, APOF, AZGP1, IGLV5-39, H2AC8, LMNA, PLTP, SERPINA11, CKB, ECM1, VNN1, LTA4H, and GXYLT2.

The validation results revealed that CKB(Fig. 3D) and YWHAZ(Fig. 3E) were significantly up-regulated in the disease group of the validation cohort, with an ROC curve indicating a predictive value of 0.764 for hypertension. Additionally, C8A, AZGP1, and APOF showed significant down-regulation. KEGG enrichment analysis highlighted the involvement of differential proteins in pathways such as Neutrophil extracellular trap formation, Systemic lupus erythematosus, Amoebiasis, Focal adhesion, and Complement and coagulation cascades(Fig. 3G). Differential expression protein-protein interaction (PPI) network analysis (Fig.3H). These proteins were validated in 60 participants in the testing phase. The validation results revealed that CKB and YWHAZ were significantly upregulated in the hypertension group, and the ROC curves showed that these two markers had a predictive value of 0.764 for hypertension (Fig. 3I). C8A, AZGP1, and APOF were significantly downregulated.

Joint analysis identified two important common pathways: arachidonic acid metabolism and pathogenic *Escherichia coli* infections (Fig. 4A). Association analysis of differentially expressed metabolites with differentially expressed proteins was performed (Fig. 4B). Association analysis of differentially expressed metabolites with biochemical indicators is presented in Fig. 4C. Machine learning models were used to analyze metabolic and protein markers. Two validated upregulated proteins, CKB in combination with YWHAZ, reached an AUC of 0.764 (0.585–0.944), and the five most upregulated metabolites, pseudopelletierine, plastoquinone 3, PI (16:0/16:0), and caffeine in combination with 2'-O-methylcajanone reached an AUC of 0.871 (0.738–1.000), and the seven substances (CKB, YWHAZ, pseudopelletierine, plastoquinone 3, PI (16:0/16:0), caffeine, and 2'-O-methylcajanone) reached an AUC value of 0.982 (0.949–1.000) (Fig. 4D).

4. Discussion

In the study of hypertension omics in plain regions, omics technologies—such as genomics, transcriptomics, proteomics, metabolomics, and glycomics—have been widely applied to uncover disease mechanisms and demonstrate significant practical applications. These include developing diagnostic tools, predicting risks, advancing personalized medicine, and monitoring treatments. By integrating multi-omics data, novel biomarkers can be identified, enhancing diagnostic accuracy and improving the effectiveness of early intervention[23]. Proteomics analysis of urine or plasma samples identifies hypertension-associated protein biomarkers such as kalirin and chromodomain-helicase-DNA-binding protein 7 (CHD7), which correlate with endothelial dysfunction in hypertensive patients and are utilized to develop diagnostic panels for renal complications[24]. Genomics identifies blood

pressure-associated single-nucleotide polymorphisms (SNPs) through genome-wide association studies (GWAS) and polygenic risk scores (PRS), such as rs9349379 in PHACTR1 and UMOD gene variants. Individuals with high PRS exhibit a 2.3-fold increased risk of hypertension and an onset accelerated by 10.6 years. These findings have been translated into risk assessment tools supporting lifestyle interventions, improving hypertension control rates, and reducing the public health burden[25–27].

The study is the first to employ a pilot multi-omics approach in investigating hypertension among Tibetan individuals residing at high altitudes (above 4,500 m). In the metabolomics and proteomics analyses of this study, only one significantly downregulated differential metabolite (PC(P-18:1(9Z)/16:0)) and one differentially expressed protein (apolipoprotein F, APOF) were identified after false discovery rate (FDR) correction (FDR < 0.05). The limited nature of these differential findings may stem from the following factors: 1. Biological variability arising from individual genetic backgrounds and specificities. 2. Small sample size, which constrained the statistical power of tests, particularly resulting in a significant reduction in the number of significantly different proteins after false discovery rate (FDR) correction[28]. 3. Due to insufficient health awareness among residents, the lack of detailed records regarding the timing of initial hypertension diagnoses may introduce heterogeneity in disease progression[29]. To further identify potential differentially expressed proteins and reduce the risk of missing true positive results, we incorporated findings based on uncorrected significance thresholds ($P < 0.05$) into subsequent exploratory analyses. This approach aims to uncover additional potential biological markers and provide candidate targets for future research[30].

It identifies common pathways with hypertension in lower-altitude regions, particularly focusing on lipid metabolism pathways[31]. This research highlights the significance of signal transduction pathways in the development of hypertension in plateau areas, with YWHAZ emerging as a potential novel biomarker. Non-target metabolomics analysis revealed disturbances in lipid metabolism, specifically glycerophospholipids and fatty acyl substances, during the onset of hypertension in plateau environments. Previous studies have shown that lipoproteins can induce the production of vascular reactive oxygen species (ROS), which act as signaling molecules in the cardiovascular system and are linked to endothelial dysfunction, hypertension, and atherosclerosis[32]. Further investigation identified the arachidonic acid metabolism pathway and the linoleic acid metabolism pathway as key up-regulated pathways in hypertension. Moreover, serum metabolites PI(16:0/16:0), Caffeine, Plastoquinone 3, 2'-O-Methylcajanone, and Pseudopelletierine were identified as biomarkers for hypertension in plateau areas, with a combined AUC of 0.871 (0.738–1.000). Notably, PI(16:0/16:0) is a phosphatidylinositol, an important lipid that serves as a major source of

arachidonic acid, essential for the synthesis of arachidonic acids such as prostaglandins[33]. Studies have shown that the enzyme activity and protein expression of key enzymes involved in arachidonic acid metabolism, cytochrome P450 (CYP) 4A and soluble epoxide hydrolase (sEH), are inhibited under the influence of antihypertensive drugs[34].

The study highlights the upregulation of metabolite PI (16:0/16:0) and arachidonic acid metabolism in individuals with hypertension living in plateau areas, underscoring the significant role of abnormal lipid metabolism in the development of hypertension. Proteomics analysis revealed 29 up-regulated proteins and 32 down-regulated proteins that were markedly different. The combination of CKB, YWHAZ, C8A, AZGP1, and APOF showed an AUC of 0.841 (0.659--1) in diagnosing hypertension. Specifically, the up-regulated CKB and YWHAZ proteins can serve as valuable biomarkers for hypertension in plateau regions, with an AUC of 0.764 (0.585–0.944). These serum proteins are implicated in the pathogenesis of hypertension. CKB, a creatine kinase, is involved in essential high-energy processes in the body such as sodium retention, cardiovascular function, arterial remodeling, and thermogenesis, potentially contributing to increased blood pressure[35,36]. Furthermore, CKB is associated with Arginine and proline metabolism. Studies have shown elevated levels of proline and tyrosine in hypertensive patients, with proline being a precursor of arginine. Notably, meta-analysis has linked increased serum CK concentration with elevated blood pressure and hypertension[37–39]. Research has also demonstrated that the CK competitive inhibitor GPA can reduce both systolic and diastolic blood pressure in hypertensive rats[40].

The hypoxic and cold environment in plateau areas can increase the body's need for heat production, potentially impacting cardiovascular health significantly. This study identified 19 differential proteins, including IGHV3-15, IGHV3-74, IGHV1-18, and others, involved in signal receptor binding within the body. Signal receptor binding emerged as a critical molecular function of these proteins. Furthermore, this study discovered, for the first time, an increased expression of YWHAZ in the serum of patients with hypertension in plateau areas. YWHAZ, a key protein in cell signal transduction, is involved in various cellular processes such as autophagy, apoptosis, and cell cycle regulation[41,42]. YWHAZ's involvement in the PI3K-Akt signaling pathway, known for its role in hypertension and hyperlipidemia, suggests its significance in the development of hypertension in plateau areas[43–47]. Additionally, the study confirmed the down-regulation of three proteins - C8A, AZGP1, and APOF. C8A, a component of the complement membrane attack complex, is linked to cell lysis and inflammation, potentially contributing to hypertension-related damage[48,49]. AZGP1, a glycoprotein associated with obesity, has been shown to correlate with elevated blood pressure[50]. APOF, crucial in lipid metabolism, may also play a role in the pathogenesis of hypertension[51]. Further speculation suggests that CKB is functionally associated with the body's energy expenditure processes, while

YWHAZ demonstrates potential roles in cellular signaling pathways; both are considered noteworthy upstream events in the development of hypertension in plateau areas.

Through bioinformatics analysis and verification, the AUC of seven serum proteins and metabolites to jointly diagnose hypertension was 0.982: CKB, YWHAZ, PI(16:0/16:0), Caffeine, Plastoquinone 3, 2'-O-Methylcajanone, and Pseudopelletierine (0.949–1.000), which is of great value for improving the accuracy of diagnosis and prediction of hypertension in the original region.
This study has several innovations. First, serum proteomics and metabolomics were used for the first time to analyze the pathogenesis of hypertension in people living in plateau areas. Second, it was discovered for the first time that signal transduction-related pathways and lipid metabolism pathways are important for the occurrence of hypertension in plateau areas. Third, YWHAZ is reported for the first time as a candidate biomarker for hypertension in plateau areas. However, this study has some limitations: the differential protein was only verified internally by PRM technology, and the sample needs to be expanded and external validation will be carried out in the future.

5. Conclusion

This study conducted a comprehensive metabolomic and proteomic analysis of hypertensive patients in plateau areas above 4500m, revealing the key pathways and possible biomarkers for the occurrence of hypertension in Tibetan people who have lived on the plateau. This study provides a basis for studying high-altitude conditions and understanding the pathogenesis of hypertension in the population.

DATA AND MATERIALS AVAILABILITY STATEMENT

All data associated with this study are present in the paper. The raw metabolomics data generated in this study are stored in the OMIX database at the National Center for Biological Information (accession number: OMIX005362). Raw proteomics data are stored in the iPROX database (accession number: IPX0007730001). We uphold the principles of open science and encourage collaborative exploration for legitimate research purposes. To ensure ethical compliance and protect privacy, data access requires prior approval through an application process. Researchers interested in utilizing the data should contact the corresponding author (kelsangnorbu@hotmail.com) with a clear statement of research objectives and a detailed data usage plan. We will evaluate each request and grant access to approved researchers. We appreciate your interest in this study and hope these data will advance further scientific discoveries.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS STATEMENT

Luobu Gesang contributed to the study’s conceptualization, funding acquisition, project administration, supervision, and specifically, the critical review of this article. Ju Huang and Danzeng Zhuoga conducted data curation, formal analysis, investigation, methodology, and the original draft writing. Bai Ci and Yangzong Suona assisted with research data, original draft writing, and gave a lot of valuable opinions. Bai Ci, Zhuoma Ciren, Chunyan Yuan, and Panduo Zhuoma contributed to the investigation and resources of study materials provision. Rui Zhang, Yangjin Baima, Yuansheng Wang, Zhuoma Pubu, Zhuoga Lamu, and Wangjie Suolang contributed to the Investigation of data collection.

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Figure legends

Fig. 1 Study design summary. Participants (n = 90) were screened, including 45 healthy controls (HC) and 45 patients with hypertension. Of these, 60 patients were included in the metabolomics study, 30 in the proteomics discovery phase, and 60 in the testing phase. Serum was collected for proteomics and metabolomics training.

Fig. 2 Metabolomics results summary. (A) Orthogonal partial least squares-discriminant analysis (OPLS-DA) score plot, (B) differential metabolite clustering heatmap, (C) differential metabolite volcano plot, (D) PI (16:0/16:0), caffeine, plastoquinone 3, 2'-O-methylcajanone, pseudopelletierine joint receiver operating characteristic (ROC) analysis, (E) Kyoto Encyclopedia of Genes and Genomes (KEGG) classification, (F) enrichment analysis KEGG pathway bubble plot, (G) network diagram, (H) random forest analysis, and (I) caffeine expression box plot.

Fig. 3 Proteomics results summary. (A) Clustering heat map of differentially expressed proteins, (B) volcano map of differentially expressed proteins, (C) clusters of orthologous genes (COG) analysis of differentially expressed proteins, (D) creatine kinase b (CKB) expression box plot, (E) tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) expression box plot, (F) gene ontology (GO) annotation enrichment analysis, (G) KEGG analysis, (H) differentially expressed protein-protein interaction (PPI) network analysis, (I) combined receiver operating characteristic (ROC) analysis of CKB and YWHAZ.

Fig. 4 Joint metabolomic-proteomic analyses results summary. (A) Metabolomic and proteomic common pathways, (B) association analysis of differentially expressed metabolites with differentially expressed proteins, (C) association analysis of differentially expressed metabolites with biochemical indices, (D) joint receiver operating characteristic (ROC) analysis of creatine kinase b (CKB), tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ), pseudopelletierine, plastoquinone 3, PI (16:0/16:0), and caffeine combined ROC analysis with 2'-O-methylcajanone.