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Urinary Proteomic Characteristics of Hyperuricemia and Their Possible Links with the Occurrence of Its Concomitant Diseases

Shuai Huo,[¶] Hongxin Wang,[¶] Meixia Yan, Peng Xu, Tingting Song, Chuang Li, Ruimin Tian, Xiaoling Chen, Kun Bao, Ying Xie, Ping Xu, Weimin Zhu, Fengsong Liu, Wei Mao,* and Chen Shao*

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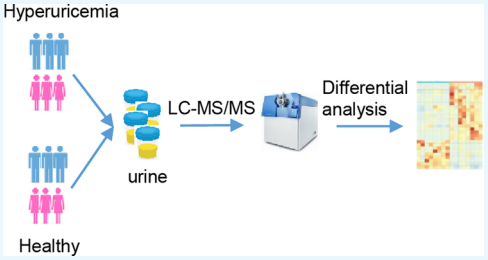
ABSTRACT: Hyperuricemia (HUA), a chronic disease caused by metabolic disorders of purine, is often accompanied by other diseases such as gout, type 2 diabetes mellitus (T2DM), and hyperlipidemia. However, little is known about the relationship between HUA and these diseases on the protein level. We performed label-free liquid chromatography MS/MS spectrometry analysis of urine samples from 26 HUA patients and 25 healthy controls, attempting to establish the possible protein links between HUA and these diseases by profiling urine proteome. A total of 2119 proteins were characterized in sample proteomes. Among them, 11 were found decreased and 2 were found increased in HUA samples. Plausible pathways found by enrichment analysis of these differentially expressed proteins (DEPs) include the processes for insulin receptor recycling and lipid metabolism, suggesting potential links between HUA and T2DM and hyperlipidemia. The abundance changes of three key proteins (VATB1, CFAD, and APOC3) involved in these processes were validated by enzyme-linked immunosorbent assay (ELISA). In conclusion, our result provides proteomic evidence, for the first time, that the aberrant pathways enriched by described key DEPs are closely related to the incidence of HUA and its concomitant diseases.



INTRODUCTION

Hyperuricemia (HUA) is a chronic disease caused by metabolic disorders of purine.[1] The incidence of HUA is growing rapidly worldwide, especially in developed countries and regions. Recent studies suggested that factors such as sex, age, ethnicity, specific genetic mutation, dietary habit, and environmental factors contribute to this increasing prevalence.[2,3] HUA is easily ignored in the early stage due to the lack of symptoms until gouty arthritis and renal tophi occur.

HUA is known associated with higher incidences of other chronic and metabolic diseases, such as hyperlipidemia, type 2 diabetes mellitus (T2DM), hypertension, major adverse cardiovascular events, and chronic kidney disease.[4−6] Until now, little is known about the relationship between HUA and these diseases. HUA has been confirmed as an independent risk factor for the occurrence and development of T2DM. Research showed that the risk of occurrence of T2DM was increased by 17% with every 60 μmol/L serum uric acid (UA) increase.[7] Studies also showed that HUA contributed to the progression of diabetic kidney disease in T2DM, as well as a significant increase in the risk of diabetic vascular complications.[8,9] Previous experimental studies suggested several possible mechanisms for the relationships between HUA and T2DM.[10−12] Among them, the leading hypothesis was that the elevated serum UA level imbalanced proinflammatory endocrine and/or reduced endothelial nitric oxide bioavailability, which subsequently resulted in insulin resistance. Another possible mechanism for HUA/T2DM link was that intracellular urate was shown to promote hepatic gluconeogenesis by activating AMPD and inhibiting AMPK.[13]

Serum uric acid (UA) level was also reported to be positively associated with an elevated level of total cholesterol, triglyceride, and low-density lipoprotein cholesterol.[14] Apolipoproteins were reported to play a key role in the pathophysiological process of lipid abnormalities among HUA patients.[15] This process has been demonstrated to be mediated by polymorphisms in the apolipoprotein APOA1-APOC3-APOA4 gene cluster.[16,17]

With the development of high-throughput experimental technologies, holistic analysis approaches have been applied to the studies of HUA and gout. A number of genes associated with urate transporting and interacting proteins were identified in the genome-wide association studies.[18−22] Metabolomics studies have discovered several dipeptides, essential amino acids, steroid hormones, and their metabolic pathways as important regulators of serum UA.[23,24] And inflammation induced by the interleukin 6/signal transducer and activator of transcription 3 signaling pathway participated in hyperuricemia-induced kidney injury.[25] Complement C3, haptoglobin, complement C4, and apolipoprotein A1 were significantly higher expressed serum proteins in

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Table 1. Basic Characteristics of the Individuals[a]

		control (n = 25)	hyperuricemia (n = 26)	T or $\chi 2$	p value
age (years)		39.16 ± 12.43	37.85 ± 10.64	−0.406	0.686
gender (male)		20(80.0)	20(76.9)	0.071	0.789
serum uric acid (μmol/L)	male	362.50 ± 51.04	508.85 ± 82.02	6.775	0.000
	female	254.40 ± 85.76	483.67 ± 175.21	2.656	0.026
urea nitrogen (mmol/L)		4.70 ± 1.15	4.70 ± 0.92	0.002	0.998
serum creatinine (μmol/L)		85.84 ± 15.45	85.96 ± 16.64	0.027	0.979
ALT (U/L)		24.24 ± 12.55	25.08 ± 12.31	0.240	0.837
AST (U/L)		21.84 ± 5.74	20.58 ± 4.99	−0.839	0.405
total protein (g/L)		75.87 ± 3.27	77.95 ± 4.22	1.968	0.055
albumin (g/L)		49.90 ± 2.52	49.45 ± 1.95	−0.705	0.484
total cholesterol (mmol/L)		4.32 ± 0.66	4.49 ± 0.61	0.992	0.326
triglyceride (mmol/L)		1.08 ± 0.40	1.29 ± 0.45	1.787	0.08
LDL cholesterol (mmol/L)		2.64 ± 0.60	2.87 ± 0.59	1.356	0.181
HDL cholesterol (mmol/L)		1.34 ± 0.37	1.21 ± 0.43	−1.147	0.257
fasting blood glucose (mmol/L)		4.84 ± 0.59	4.95 ± 0.32	0.823	0.415

[a]Values for continuous data are mean ± SD; for categorical data are count (percentage).

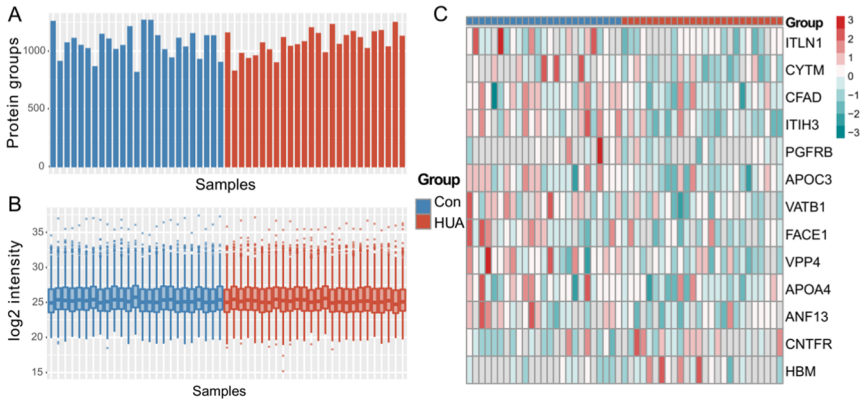

Figure 1. General information of proteome results. HUA, hyperuricemia group; Con, control group. (A) Number of proteins identified in each sample. (B) Abundance distribution. (C) Heatmap of all differentially expressed proteins (DEPs) in HUA. The color scale indicates the z-score-transformed abundance data of each DEP. Gray indicates a missing value in quantification.

the Uygur hyperuricemia group than in the normal control group by two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF-MS).[26]

Urine is one of the most valuable clinical samples for the discovery of biomarkers. Compared with blood and tissue fluid, urine protein detection has the following advantages. First, urine is a kind of sample that can be collected noninvasively and repeatedly. Second, urine contains proteins that are filtered from plasma and secreted from the urinary system and, therefore, can reflect changes in the whole body system.[27,28] Thirdly, lots of studies have shown that urine is more suitable for mass spectrometry analysis than blood,[29−31] as it is less affected by the high-abundance proteins such as albumin and immunoglobulins. In addition to the applications in disease diagnosis, the urine proteome might offer new insight into the pathogenesis of hyperuricemia.

Mass spectrometry (MS)-based proteomics has become a powerful tool that can routinely detect and quantify thousands of proteins, providing great opportunities in the discovery of biomarkers for a broad range of potential clinical applications. In this study, we performed label-free liquid chromatography−tandem mass spectrometry (LC−MS/MS) analysis on the urine samples from patients with HUA and healthy controls to characterize the urinary proteomic features of HUA and to establish possible protein links between HUA and its concomitant diseases.

RESULTS

Patient Group. Twenty-six patients (20 males and 6 females) aged between 22 and 71 years (mean age, 37.9 years) with HUA were enrolled in the study as the HUA group, with 25 normal volunteers (20 males and 5 females, aged between 24 and 70 years with a mean age of 39.2 years) recruited as the control group. No statistical differences were found in the distribution of gender and age between these two groups. Individuals in both study groups have been confirmed clinically without other diseases such as hyperlipidemia, diabetes, and liver and kidney diseases. Basic information of enrolled individuals is given in Table 1. Their detailed clinical data are provided in Table S1.

General Information of Proteome Results. A total of 2119 proteins were identified and quantified by the label-free LC−MS/MS approach with MaxQuant software (version 1.6.2.10).[32] Both peptide-spectrum match (PSM) and protein false discovery rate (FDR) were set to 0.01. Also, the minimum number of unique peptides for each protein was set to 2. As shown in Figure 1A,1B, similar numbers of proteins were identified between all samples in the two groups (Figure 1A), and protein abundance distributions among samples were nearly

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Table 2. Differentially Expressed Proteins in Hyperuricemia Compared to Normal Control[a]

symbol	accession	description	*p* value	fold change	change
ITIH3	Q06033	inter-α-trypsin inhibitor heavy chain H3	0.0015	0.5404	downregulation
CFAD	P00746	complement factor D	0.0292	0.5322	downregulation
APOC3	P02656	apolipoprotein C-III	0.0140	0.5883	downregulation
VATB1	P15313	V-type proton ATPase subunit B kidney isoform	0.0052	0.5990	downregulation
CYTM	Q15828	cystatin-M	0.0112	0.4469	downregulation
FACE1	O75844	CAAX prenyl protease 1 homolog	0.0351	0.6010	downregulation
VPP4	Q9HBG4	V-type proton ATPase 116 kDa subunit a isoform 4	0.0076	0.6057	downregulation
RNF13	O43567	E3 ubiquitin-protein ligase RNF13	0.0091	0.6572	downregulation
APOA4	P06727	apolipoprotein A-IV	0.0383	0.6431	downregulation
ITLN1	Q8WWA0	intelectin-1	0.0422	0.0624	downregulation
PDGFRB	P09619	platelet-derived growth factor receptor β	0.0462	0.5782	downregulation
HBM	Q6B0K9	hemoglobin subunit mu	0.0311	8.5500	upregulation
CNTFR	P26992	ciliary neurotrophic factor receptor subunit α	0.0419	1.7430	upregulation

[a]Fold change, the fold change (HUA/control).

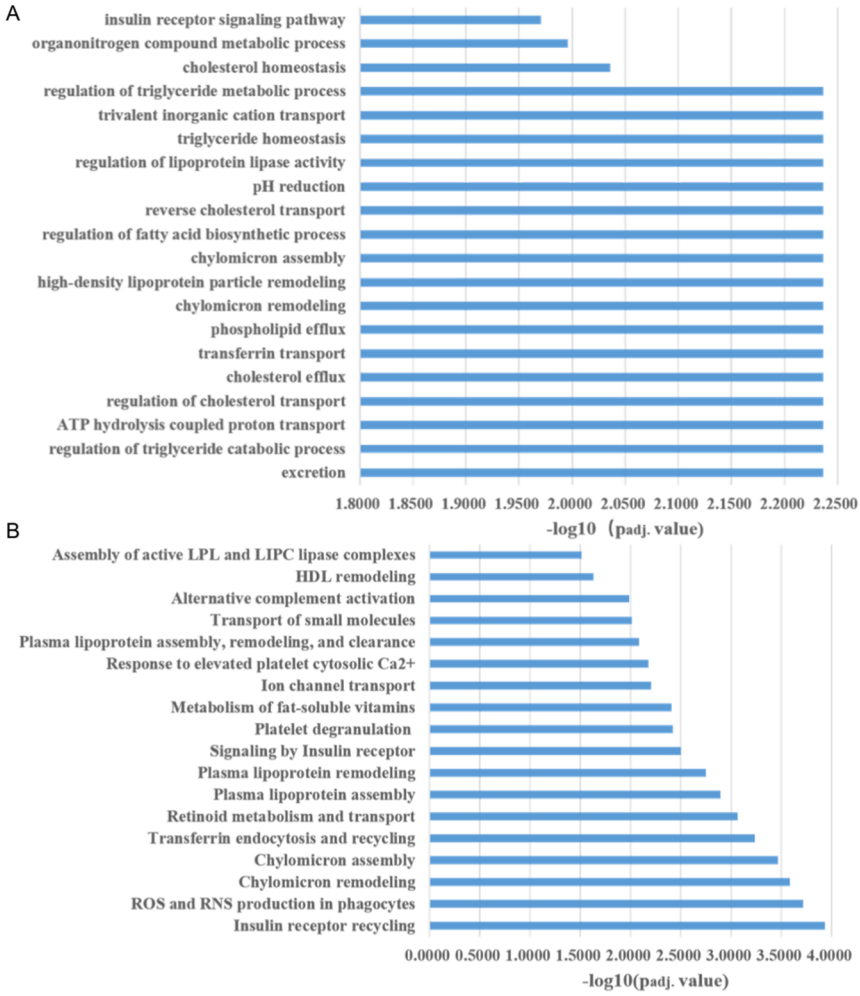

Figure 2. Functional enrichment analysis of DEPs. (A) Significantly enriched biological processes in Gene Ontology. (B) Significantly enriched Reactome pathways.

identical (Figure 1B). In total, 78.01% of proteins are classified as extracellular proteins (extracellular exosome, extracellular space, extracellular matrix, or extracellular region) in Gene Ontology (GO). To evaluate the comprehensiveness of our urinary proteomic data set, the 2119 proteins were compared with those identified in previous urinary proteomic studies on the concomitant diseases of HUA. Our data set includes 56.12% (463/825) of proteins with at least two unique peptides reported in a T2DM urinary proteome by Anh et al.,[33] 63.45% (349/550) of gene products identified in another T2DM urinary proteome by Guillén-Gómez et al.,[34] and 69.00% (512/742) proteins in a urinary proteome data set from patients with hypertension.[35] Besides, our proteome data set contains 78.95% (1579/2000) of the normal urinary proteome demonstrated in Leng et al.'s study,[36] which employed very similar sample preparation and proteomic analysis approaches as in this study.

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These observations indicate good technical reproducibility and validity of our proteomic analysis. A total of 1102 proteins with valid abundance values in at least 50% of the samples in a certain group were selected for further analysis (Table S2).

Differential Urinary Proteins in Hyperuricemia. Thirteen DEPs were identified between HUA and Con groups by the *t*-test (p value <0.05, fold change ≥1.5, Table 2). Eleven of them were downregulated and two were upregulated in the HUA group (Figure 1C).

Gene Ontology (GO) Enrichment and Pathway Analysis. GO enrichment analysis result is outlined in Table S3. Enriched biological processes are mainly involved in lipid metabolism, adenosine 5′-triphosphate (ATP) hydrolysis coupled proton transport, and the insulin receptor signaling pathway (Figure 2A). Enriched molecular functions include cholesterol binding, enzyme inhibitor activity, proton transmembrane transporter activity, etc. Pathway analysis using Reactome (Figure 2B and Table S4) also showed enriched pathways of insulin receptor recycling as well as those related to lipid metabolism, such as plasma lipoprotein and chylomicron assembly and remodeling. Besides, pathways of platelet degranulation, metabolism of fat-soluble vitamins, and others were also enriched among the DEPs.

Note that the results of GO and pathway analyses might be influenced by the small number of DEPs, and the aforementioned significantly enriched GO terms and pathways need to be further validated, either by a deeper proteomic analysis with expanded sample size or by other mechanism studies.

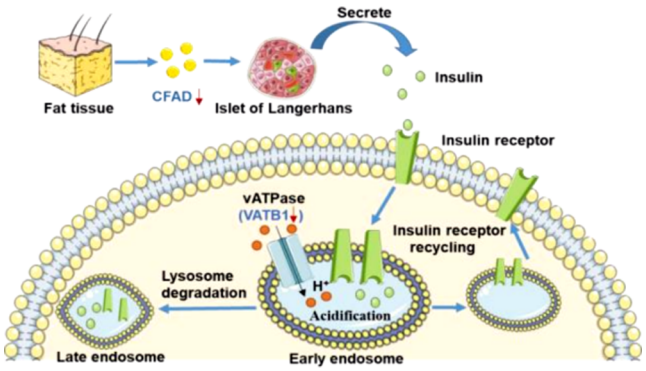

Figure 3. Schematic diagram of possible functional mechanisms of insulin resistance induced by VATB1 and CFAD. DEPs are indicated with blue characters and red arrows. Decreased VATB1 may lead to insufficient and untimely acidification of the endosomal lumen, which consequently disturbs the insulin receptor recycling and delays its binding to insulin, leading to insulin resistance. Downregulated CFAD may be involved in the development of T2DM by decreasing insulin secretion.

Validation of DEPs. Three DEPs with interesting molecular functions that might be linked to the pathogenesis of the HUA-associated diseases were validated by enzyme-linked immunosorbent assay (ELISA). Samples used for validation include 17 HUA and 24 control samples used in the MS analysis, and 8 HUA and 7 control samples from another cohort. A one-sided *t*-test was performed on the logarithm-transformed protein-to-creatine ratio of the proteins between the control group and the HUA group. Downregulation of APOC3 (p = 0.0137) CFAD (p = 0.0418), and VATB1 (p = 0.0885) in the HUA group was validated at the significance level of 0.1. The results are shown in Figure 4.

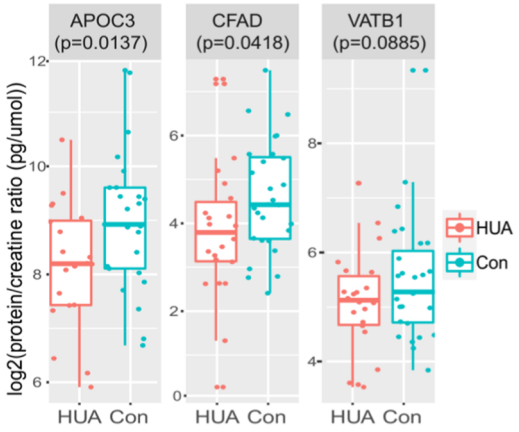

Figure 4. Validation of five DEPs by ELISA Three DEPs with interesting molecular functions that might be linked to the pathogenesis of the HUA-associated diseases were validated by ELISA. The one-sided *t*-tests were performed on the logarithm-transformed protein-to-creatine ratio of the proteins between the control and the HUA group.

DISCUSSION

HUA is an independent risk factor for the occurrence and development of many diseases, such as gout, hyperlipidemia, T2DM, hypertension, cardiovascular disease, and chronic kidney disease. However, the specific pathogenesis is still not clear. In this study, we identified a total of 13 proteins that had significant abundance changes in the urine of HUA patients. Since a large proportion of urinary proteins come from renal filtration of plasma proteins, it can be assumed that changes of these proteins in the urine can reflect their corresponding changes in the blood. We discussed the associations between these altered proteins and reported potential molecular changes of some diseases that are closely related to HUA in the following subsections, showing that urinary proteomics may help explain their pathogenesis. Some of these urinary proteins may serve as noninvasive biomarkers for monitoring and early detection of some of these diseases in the future.

VATB1 and CFAD May Promote the Occurrence of T2DM by Affecting Insulin in HUA Patients. Previous studies have confirmed that HUA is an independent risk factor for T2DM and urate-lowering therapy can notably ameliorate insulin resistance.[37,38] VATB1, together with another downregulated protein VPP4, is involved in the insulin receptor recycling pathway. The two proteins are mainly expressed in distal renal tubular epithelial cells, which is one of the main sites of insulin degradation. They are components of the proton channel of V-ATPase, which could acidify the eukaryotic endosomal lumen by pumping H^+ into it from the cytoplasm.[39] Physically, the acidification of the endosomal lumen triggers insulin to dissociate from its receptor and be degraded in the endosome lumen. The insulin receptor is then recycled into the plasma membrane after being dephosphorylated.[39] We hypothesize that decreased VATB1 and VPP4 may lead to insufficient and untimely acidification of the endosomal lumen, disturbing the insulin receptor recycling and delaying its binding to insulin, which is a crucial feature of insulin resistance (Figure 3).

CFAD also has a close relationship with T2DM. CFAD[40] is a positive regulator of the alternative complementary pathway by catalyzing the cleavage of complement factor B, a rate-limiting protease of complement activation. Historically, CFAD was known as adipsin which can regulate energy homeostasis and

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systemic metabolism. Research has demonstrated that T2DM patients with β cell failure are deficient in CFAD and CFAD acts, at least, in part, via C3a to potentiate insulin secretion.[41] Decreased urinary CFAD in HUA patients may be involved in the development of T2DM by decreasing insulin secretion.

APOC3 May Participate in the Pathogenesis of Hypertriglyceridemia in HUA Patients. Increased serum UA is a well-known risk factor for hyperlipidemia, and the serum triglyceride level has the strongest association with the serum UA level.[42] Some biological processes and pathways related to lipid metabolism were significantly enriched in patients with HUA, such as regulation of the triglyceride catabolic process (Figure 2A), plasma lipoprotein and assembly, and remodeling (Figure 2B). APOC3 is synthesized in the liver and intestine and is the most abundant C apolipoprotein in human plasma.[43] It was reported that APOC3 could change triglyceride metabolism by affecting the activity of lipoprotein lipase (LPL) and clearance of triglyceride-rich lipoproteins (TRLs).[44] However, it is still difficult to speculate how APOC3 participated in the development of hypertriglyceridemia in HUA patients based on the limited understanding of the molecular mechanisms of HUA. Future studies are needed to first validate their downregulation in blood and the corresponding organs and then to discover their roles in the pathogeneses of HUA-related hypertriglyceridemia.

Possible Relationships between HUA and Other DEPs. The positive correlation between serum uric acid and hemoglobin levels has long been known.[45−48] HUA is also commonly occurred in patients with polycythemia. Increased red cell degradation in subjects with increased red cell mass may be the cause of this correlation.[48]

In vitro study in vascular smooth muscle cells showed that uric acid could induce proliferative pathways through its phosphorylation. This process is thought to play a crucial role in the development of cardiovascular diseases.[49]

Several proteins that are known factors of HUA-induced injuries have also been detected in our study. HUA could activate the renin−angiotensin system (RAS), which is closely related to metabolic syndromes and cardiovascular and kidney diseases.[50,51] Two key factors in this system, angiotensinogen and angiotensin-converting enzyme (ACE), were quantified in our study. Our proteomic data set also includes MMP7 and fibronectin, which associate with HUA-induced renal fibrosis,[50] as well as CXCL-12, an indicator of inflammation.[50] However, these proteins exhibited no significant differences between HUA and control groups (Table 3). The fact that all patients were free from hypertension and any renal diseases at the time of urine collection may be the explanation of these observations.

Table 3. Known Proteins Associated with HUA-Induced Disease

symbol	accession	description	p value	fold change
AGT	P01019	angiotensinogen	0.0983	0.7488
ACE	P12821	angiotensin-converting enzyme	0.9565	1.051
FN	P02751	fibronectin	0.321	1.5071
MMP7	P09237	matrix metalloproteinase-7	0.6849	1.3176
CXCL-12	P48061	C-X-C motif chemokine 12	0.7281	0.8842

CONCLUSIONS

In conclusion, HUA is a systemic metabolic disease that may have impacts on various systems of the human body. Taking advantage of advanced proteomic techniques, the present study characterized the urinary proteomic features of HUA and explored the possible protein links between HUA and its concomitant diseases. Specifically, we identified and validated three key downregulated expressed proteins: VATB1 and CFAD, which are associated with insulin resistance and regulation of insulin secretion, and APOC3, which is related to triglyceride metabolism. We hypothesize these candidate proteins may be related to the pathogenesis of T2DM and hypertriglyceridemia associated with HUA.

These proteins may help us to monitor and detect the subsequent occurrence of the concomitant diseases in patients with HUA timely and provide evidence for intervention. Other DEPs may give us more clues for HUA-related diseases. Further work to verify the above DEPs in blood samples from HUA patients will help us to reveal the detailed relationships between HUA and its concomitant diseases.

METHODS

Patients. Volunteers were divided into the control group and experiment group. In the control group, there were 25 healthy people (20 males and 5 females) aged between 24 and 70 years. The HUA group consisted of 26 patients with HUA (20 males and 6 females) aged between 22 and 71 years. Both groups did not include the cases associated with other common diseases, such as hyperlipidemia, diabetes, liver, and kidney diseases. The diagnosis criteria of HUA patients are as follows: fasting serum uric acid level $\geq$420 μmol/L for males and $\geq$360 μmol/L for females, associated with gout or not. All volunteers with informed consent documents were from the physical examination population in the physical examination center of Guangdong Provincial Hospital of Chinese Medicine. The study was approved by the Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine.

Sample Collection and Storage. Approximately 10 mL of midstream urine from each volunteer was collected into one sterile urinary sediment tube according to the criteria of morning urine retention. All collected urine samples were stored at −80 °C and left standing.

Sample Preparation. One milliliter of urine sample was centrifuged at 176 000g for 70 min at 4 °C to collect the pellets. We utilized a previously described method[52] with modifications to remove uromodulin (UMOD). Briefly, resuspension buffer (50 mM Tris, 250 mM sucrose, pH 8.5) and dithiotheitol (DTT) were added to the pellets and the suspension was then heated at 65 °C for 30 min. Wash buffer (10 mM tetraethylammonium (TEA), 100 mM NaCl, pH 7.4) was added to the pallets, and second ultracentrifugation was carried out at 176 000g for 40 min. Then, the supernatant was discarded. NH_4HCO_3 (50 mM) was added to the precipitate and heated at 95 °C for 3 min. Samples were then digested with trypsin overnight at 37 °C. Then, the supernatant was extracted by centrifugation with 100% ACN at 10× 1000g for 5 min and dried in a vacuum concentrator (Thermo Scientific); the resulting peptides were used for mass spectrometry analysis.

Liquid Chromatography−Tandem Mass Spectrometry. Peptides were loaded onto a trap column (100 μm × 2 cm, homemade; particle size, 3 μm), separated by a homemade silica microcolumn (150 μm × 12 cm, particle size, 1.9 μm) with a

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gradient of 5−35% mobile phase B (80% acetonitrile and 0.1% formic acid) at a flow rate of 800 nL/min for 30 min. The analysis was performed on a Q Exactive HF mass spectrometer (Thermo Fisher Scientific, Rockford, IL) connected to an Easy-nlc 1200 System (Thermo Fisher Scientific). Peptides were analyzed by an Orbitrap mass analyzer with a resolution of 60 000 in full scan mode and 15 000 in MS/MS mode. The MS/MS analysis was performed in a data-dependent mode. One full scan was followed by up to 15 data-dependent MS/MS scans with higher-energy collision dissociation (normalized collision energy of 27%). The dynamic exclusion time was set as 15 s.

Mass Spectrometry Data Analysis. The mass spectrometry raw files were analyzed with MaxQuant (version 1.6.2.10). A total of 51 raw files were analyzed together to exploit the match-between-run algorithm, which enables the identification of peptides that were not selected for fragmentation in one run by checking whether these peptides were sequenced in another run.[53] We used the Andromeda search engine[54] to search the detected features against the human Swiss-Prot reference proteome from Uniprot (downloaded on 18 March 2019, 20251 proteins). Only tryptic peptides that were at least seven amino acids in length with up to two missed cleavages were considered. N-acetylation of proteins' N-termini and oxidation of methionine were set as variable modifications. The first search peptide tolerance was set to 4.5 ppm for the main search peptide tolerance for the Q Exactive data at the MS level and 0.05 Da at the MS/MS level. A false discovery rate (FDR) of 1% was imposed for peptide-spectrum matches (PSMs) and protein identification using a target−decoy approach. The number of minimal unique peptides was set to 2. To normalize the differences in peptide loading amounts, relative quantification was performed using the default parameters of the MaxLFQ algorithm[55] with the minimum ratio count set to 2. For all other search parameters, the default settings were used.

The "proteinGroups.txt" file produced by MaxQuant was further analyzed in the R language (R version 3.5.1). Proteins from the reverse database and contaminants were removed.

Statistical Analysis. A two-sided *t*-test was performed on the logarithmized LFQ intensities with a *p*-value cutoff of 0.05 and fold change ≥1.5.

Protein Gene Ontology and Pathway Analysis. Cellular component annotation for identified proteins was performed using DAVID (https://david.ncifcrf.gov/) with the GOTERM_CC_DIRECT database. To further research the biological functions of the DEPs in hyperuricemia compared to normal control, functional annotation was performed. Gene Ontology enrichment analysis was performed using STRING (version 11.0, https://string-db.org/), an open-source software tool, for general characterization of properties of the proteins in the data set. False discovery rate (FDR) <0.05 was set as the cutoff of significance. Pathway analysis was performed using Reactome (version 70, https://reactome.org/), an open-source software tool. *p* value <0.05 was set as the cutoff of significance.

Validation of DEPs by ELISA. First, human urine samples were centrifuged at 2000*g* for 20 min. VATB1 was detected by HUMAN ELISA kits (Quanzhou Ruixin Biotechnology Co., Ltd., Quanzhou, China) according to the manufacturer's instructions. APOC3 and CFAD were detected by HUMAN ELISA kits (Abcam, Cambridge, U.K.) according to the manufacturer's instructions. In brief, the samples and standards were added to appropriate wells and then the antibodies were added to all wells. The mixtures were incubated for 1 h; then, the wells were aspirated and washed with 350 μL of 1× wash buffer PT three (or five) times. The TMB development solution was added to each well and incubated for 10 (or 15) min. The stop solution was added to each well, and the absorbance at 450 nm was read on a Thermo Scientific Multiskan FC microplate reader (Thermo Scientific, Massachusetts). The concentration of urine creatinine was measured by Roche cobas 8000 (Roche, Basel, Switzerland). The final concentration of protein was corrected by the concentration of the creatinine for statistical analysis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c06229.

Detailed clinical data of all samples (Table S1), quantification information for 1102 proteins that were quantified in at least 50% of the samples in a certain group (Table S2), gene ontology analysis of DEPs in the HUA group (Table S3), pathway analysis of DEPs in the HUA group (Table S4), results of ELISA experiments (Table S5), and detailed clinical data of 15 additional samples used in the validation experiments (Table S6) (PDF)

(XLSX)

■ AUTHOR INFORMATION

Corresponding Authors

Chen Shao − *State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 102206, China;* orcid.org/0000-0002-6899-5353; Email: scshaochen@126.com

Wei Mao − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China; Department of Nephrology, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China; Guangdong Provincial Academy of Chinese Medical Sciences, Guangzhou 510006, China*; Email: maowei@gzucm.edu.cn

Authors

Shuai Huo − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China; Department of Nephrology, Henan Provincial People's Hospital, Department of Nephrology of Central China Fuwai Hospital, Zhengzhou, Henan 450003, China*

Hongxin Wang − *State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 102206, China; Key Laboratory of Zoological Systematics and Application of Hebei Province, College of Life Sciences, Hebei University, Baoding 071002, China*

Meixia Yan − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China*

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Peng Xu − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China; Department of Nephrology and Guangdong Provincial Key Laboratory of Chinese Medicine for Prevention and Treatment of Refractory Chronic Diseases, Department of Pediatrics, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China; Guangdong Provincial Academy of Chinese Medical Sciences, Guangzhou 510006, China*

Tingting Song − *State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 102206, China*

Chuang Li − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China; Department of Nephrology and Guangdong Provincial Key Laboratory of Chinese Medicine for Prevention and Treatment of Refractory Chronic Diseases, Department of Pediatrics, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China; Guangdong Provincial Academy of Chinese Medical Sciences, Guangzhou 510006, China*

Ruimin Tian − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China; Department of Nephrology and Guangdong Provincial Key Laboratory of Chinese Medicine for Prevention and Treatment of Refractory Chronic Diseases, Department of Pediatrics, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China; Guangdong Provincial Academy of Chinese Medical Sciences, Guangzhou 510006, China*

Xiaoling Chen − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China*

Kun Bao − *State Key Laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510120, China; The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China; Department of Nephrology and Guangdong Provincial Key Laboratory of Chinese Medicine for Prevention and Treatment of Refractory Chronic Diseases, Department of Pediatrics, Guangdong Provincial Hospital of Chinese Medicine, Guangzhou 510120, China; Guangdong Provincial Academy of Chinese Medical Sciences, Guangzhou 510006, China*

Ying Xie − *State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Macau (SAR) 999078, China;* orcid.org/0000-0003-3223-1797

Ping Xu − *The Second Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510405, China; State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 102206, China; Key Laboratory of Zoological Systematics and Application of Hebei Province, College of Life Sciences, Hebei University, Baoding 071002, China;* orcid.org/0000-0002-0628-6981

Weimin Zhu − *State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences (Beijing), Beijing Institute of Lifeomics, Beijing 102206, China*

Fengsong Liu − *Key Laboratory of Zoological Systematics and Application of Hebei Province, College of Life Sciences, Hebei University, Baoding 071002, China;* orcid.org/0000-0001-6634-7152

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c06229

Author Contributions

¶S.H. and H.W. contributed equally to this work.

Notes

The authors declare no competing financial interest.

Mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository[56] with data set identifier PXD016900.

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