Mass Spectrometric Study of Stone Matrix Proteins of Human Bladder Stones

Yeong-Chin Jou, Yuh-Shyan Tsai, Chiung-Yao Fang, Syue-Yi Chen, Fang-Hung Chen, Cheng-Hsing Huang, Yun-Hsuan Li, and Cheng-Huang Shen

OBJECTIVE To evaluate the mechanisms of bladder uric acid stone (BUAS) formation by analyzing BUAS stone matrix proteins, with mass spectrometry (MS).

MATERIALS AND METHODS Stone matrix proteins were extracted from 5 pure BUASs. The obtained proteins were analyzed with reverse phase liquid chromatography-tandem MS. The acquired data were investigated against a Swiss Prot human protein database, using Matrix Science Mascot. The identified proteins were submitted to UniProtKB website for gene ontology analysis to define their correlation. They were also submitted to Metacore platform and Kyoto Encyclopedia of Genes and Genomes website for pathway analysis. MS-determined protein expressions were validated by immunoblot.

RESULTS The liquid chromatography-tandem MS analysis identified 58-226 proteins in the 5 BUASs (450 proteins). Metacore software analysis suggests that inflammation might play an important role for BUAS formation. The analysis of endogenous metabolic pathways revealed that these proteins were categorized into glycerophospholipid or glycosphingolipid biosynthesis. Four of 5 identified proteins selected for validation, including uromodulin, S100P, Histone 4, and nucleophosmin, can be validated in the immunoblot data.

CONCLUSION Our results suggest that inflammatory process and lipid metabolism might play a role in the formation of BUAS. Whether these inflammatory responses are the etiology of stone formation or whether they result from local damage by stone irritation is uncertain. UROLOGY 82: 295–300, 2013. © 2013 Elsevier Inc.

Bladder stones account for 5% of urinary tract stones and are usually formed by bladder outlet obstruction, neurogenic bladder, urinary tract infection, or foreign bodies.[1] The most common type of bladder stones in adults are composed of uric acid (UA), >50%, followed by calcium oxalate, calcium phosphate, ammonium urate, cysteine, or magnesium ammonium phosphate (when associated with an infection).[2] The pathogenesis of bladder stones has not been studied as comprehensively as that of renal stones, and only limited contemporary series regarding bladder stones have been reported in previous medical studies.

Urinary stones in humans are composed of inorganic minerals and organic matrix. The organic matrix of urinary stones accounts for 2%-3% of the stone burden,[3] and proteins comprise approximately 64% of the matrix. Stone matrix proteins (SMPs) are considered to be important in stone formation by acting as a template and regulator in the formation and growth of the stone.[4] Identification of the SMPs of urinary stones might be helpful in understanding the mechanisms of stone formation, and several methods of SMP extraction have been reported.[5] With advances in proteomic studies and the application of mass spectrometry (MS), it has been possible to investigate the increasing numbers of SMPs.[6,7]

The etiologies of kidney UA stone formation are multifarious and might include impaired ammoniagenesis, insulin resistance, genetic, and idiopathic causes.[8] However, the etiology of UA urinary bladder stones in humans has not been fully analyzed. Moreover, most studies on SMPs have focused on kidney stones with few reports on bladder stones, and the use of MS for human urinary bladder stones has never been reported before. In this study, we extracted proteins from urinary bladder UA stones (the most common type of urinary bladder stone) and analyzed their characteristics by reverse phase (RP) liquid chromatography (LC) and electrospray ionization-tandem MS (MS/MS), to identify more matrix proteins and evaluate the possible mechanism of the formation of urinary bladder UA stone.

Drs. Yeong-Chin Jou and Yuh-Shyan Tsai contributed equally to this work.

Financial Disclosure: *The authors declare that they have no relevant financial interests.*
Funding Support: *This study was supported by the Chia-Yi Christian Hospital (Grant R100-17).*

From the Department of Urology, Chiayi Christian Hospital, Chia-Yi, Taiwan; the Department of Urology, College of Medicine and Hospital, National Cheng Kung University, Tainan, Taiwan; the Department of Medical Research, Chiayi Christian Hospital, Chia-Yi, Taiwan; and the Department of Laboratory Medicine, Chiayi Christian Hospital, Chia-Yi, Taiwan

Reprint requests: Fang-Hung Chen, B.S., Department of Laboratory Medicine, Chiayi Christian Hospital, 539, Chung-Hsiao Road, Chia-Yi 600, Taiwan. E-mail: cych00487@Gmail.Com

Submitted: January 28, 2013, accepted (with revisions): April 6, 2013

© 2013 Elsevier Inc.
All Rights Reserved

0090-4295/13/$36.00
http://dx.doi.org/10.1016/j.urology.2013.04.011

MATERIALS AND METHODS

Study Population

Five pure urinary bladder UA stones from 5 individuals obtained from the Institutional Review Board-approved stone bank of Chiayi Christian Hospital were selected. All the stones were collected during cystoscopic cystolithotripsy and the mineral compositions of these stones were determined by Fourier-transform infrared spectroscopy.

Protein Extraction

About 200-300 mg of stone fragments from each individual were collected and washed in deionized water, with gentle stirring to remove contaminated debris. The stone powder was suspended in Mammalian Protein Extraction Reagent (M-PER; Thermo Fisher Scientific, San Jose, CA) in a 1:2 ratio. The mixtures were homogenized with mechanical bead beater tissue homogenizer (Precellys 24 lysis/homogenizer, Bertin Technologies, France) at 5×6000 rpm for 20 seconds and then centrifuged at 14,000 *g* for 15 minutes. The supernatant containing the SMPs was stored at −80°C until proteomic analysis.

Analysis of the SMPs by LC-MS/MS

The stone proteins were reduced in 10 mM dithiothreitol for 1 hour and subsequently alkylated in 50 mM iodoacetamide for 30 minutes in darkness. The solution containing the unreacted dithiothreitol and iodoacetamide was removed by chloroform-methanol precipitation; the pellets were then suspended in 50 mM NH_4HCO_3 solution containing 0.2 μg/μL trypsin. Digestion was carried out at 37°C for 18 hours followed by the addition of one-tenth volume of 1% formic acid. The resulting solution was then used for mass spectrometric analysis. The tryptic sample was subjected to LC using an Accela 600 pump (Thermo Fisher Scientific) with a Biobasic-18 column (1 mm i.d., 150 mm in length, Thermo Scientific) via 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in 100% acetonitrile (mobile phase B), and a linear gradient from 0% to 80% mobile phase B for 105 minutes at a flow rate of 70 μL/min was applied. The peptides were analyzed under positive survey scan mode on a LTQ-Velos (Thermo Scientific) instrument. The scan range was set to m/z 300-1600 for MS and m/z 50-2000 for MS/MS. Tandem mass spectra were extracted and the charge state was deconvoluted and deisotoped by MASCOT Distiller version 2.3 (Matrix Science, London, UK). All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version Mascot), which was set up to investigate the sprot_20110407 database (selected for Homo sapiens, unknown version, 20,233 entries) assuming the digestion enzyme trypsin. Mascot was investigated with a fragment ion mass tolerance of 0.50 Da and a parent ion tolerance of 0.50 Da.

The iodoacetamide derivative of cysteine was specified in Mascot as a fixed modification. N-Succinimide of asparagine and oxidation of methionine were specified in Mascot as variable modifications.

Bioinformatic Analysis

The identified proteins were submitted for gene ontology analysis (UniProtKB; http://www.uniprot.org/uniprot/) to define their correlation. Network analyses of differentially detected proteins were performed using the MetaCore Analytical Suite (GeneGo Inc., St Joseph, MI, http://www.genego.com) via the entry website of the bioinformatics center at National Cheng Kung University. MetaCore was used to calculate the statistical significance (*P* value) according to the probability or frequency of a random set of proteins or genes from the same size as the input list.[9] The protein name, together with its frequency of detection from the present proteomic study, was uploaded into the working site of the Metacore platform. The top 10 postulated involved regulatory networks and endogenous metabolic pathways were then listed with their *P* values. The endogenous metabolic pathways were then analyzed for the identified proteins. The top 10 postulated involved endogenous pathways were submitted to the Kyoto Encyclopedia of Genes and Genomes website (KEGG, http://www.genome.jp/kegg/) for pathway analysis.

Western Blot Validation

According to the findings from MS, we selected 5 proteins, uromodulin (also called Tamm-Horsfall protein), S100P, histone 4, nucleophosmin, and clusterin for validation using Western blotting analysis in 3 stone specimens. The proteins were probed by the following primary antibodies: anti-UMOD monoclonal antibody (Abnova, Walnut, CA), anti-S100P (Cell Signaling Technology, Danvers, MA), antihistone H4 (Abcam Inc., Cambridge, MA), antinucleophosmin (Abcam Inc., Cambridge, MA), and anticlusterin α-chain (Upstate, Temecula, CA), followed by antirabbit or antimouse IgG polyclonal antibodies conjugated to peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) and detected with an enhanced OPTI-4CN Colorimetric Detection kit (Bio-Rad, Hercules, CA).

RESULTS

The LC-MS/MS analysis identified 58-226 proteins in the 5 stone samples (450 proteins). Of the 450 proteins, 21 were detected in all 5 stones and 41 were detected in 4 of the 5 stones. The origins of these proteins were determined by gene ontology analysis (Table 1). The postulated pathway associated with the protein matrix of each urinary bladder UA stone was analyzed using Metacore software, which showed that inflammation and complement systems were most likely to be involved in the formation of the stones, followed by the cytoskeleton and intermediate filament system, immune response-phagocytosis system, blood coagulation system, and response to hypoxia and oxidative stress system (Fig. 1). These results suggest that inflammation might play an important role in the formation of urinary bladder UA stones.

Endogenous metabolic pathways were also analyzed for the identified proteins. Among the top 10 postulated involved endogenous pathways (Table 2), the most significant pathway was the alpha-L-fucosyl-(1-2)-D-galactose pathway, followed by the phosphatidylcholine pathway, metabolism-prostaglandin metabolism, and 1-acyl-glycerol-3-phosphoethanolamine pathway. After investigating the KEGG website (http://www.genome.jp/kegg/) for pathway analysis, most were found to be categorized into glycerophospholipid or glycosphingolipid biosynthesis.

Of the 5 selected proteins for validation, uromodulin, S100P, and histone 4 were confirmed by immunoblotting in 2 of the 3 samples (Fig. 2). When detected by

Download English Version:

https://daneshyari.com/en/article/6167269

Download Persian Version:

https://daneshyari.com/article/6167269

Daneshyari.com