Case Report

Three cases of xanthinuria identified by gas chromatography/mass spectrometry-based urine metabolomics

Tomiko Kuhara,[1] Masahiro Tetsuo,[2] Morimasa Ohse,[1] Toshihiko Shirakawa,[3] Yumiko Nakashima,[3] Koh-ichiro Yoshiura,[4] Nagaaki Tanaka[5] and Tadashi Taya[6]

[1]Japan Clinical Metabolomics Institute, Kahoku, [2]Testuo Hospital, Minamishimabara, [3]Department of Pediatrics, Nagasaki University Hospital, [4]Department of Human Genetics, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, [5]Center for Diabetes, Endocrinology and Metabolism, Kansai Electric Power Hospital, Osaka, and [6]Taya Urology Clinic, Komatsu, Ishikawa, Japan

Abbreviations & Acronyms
AKI = acute kidney injury
AMED = Agency for Medical Research and Development
AO = aldehyde oxidase
EIAKI = exercise-induced acute kidney injuries
FEUA = fractional excretion of urate
GC/MS = gas chromatography/mass spectrometry
GEPH = gephyrin
HUS = hemolytic uremic syndrome
LC/MS = liquid chromatography/mass spectrometry
MOCOS = molybdenum cofactor sulfurase
XDH = xanthine dehydrogenase

Correspondence: Tomiko Kuhara Ph.D., Japan Clinical Metabolomics Institute, Hamakita I 13-2, Kahoku, Ishikawa 929-1174, Japan. Email: jcmi-kuhara@bell.ocn.ne.jp

How to cite this article: Kuhara T, Tetsuo M, Ohse M *et al*. Three cases of xanthinuria identified by gas chromatography/mass spectrometry-based urine metabolomics. *IJU Case Rep*. 2023; 6: 436–439.

Received 4 April 2023; accepted 6 September 2023.
Online publication 28 September 2023

Introduction: Early diagnosis of patients with urolithiasis or hypouricemia owing to inborn errors of hypoxanthine metabolism is important in preventing renal failure or drug-induced toxicity.

Case presentation: We identified three patients with xanthinuria using gas chromatography/mass spectrometry-based urine metabolomics: a 72-year-old male with bladder stone, a severe hypouricemic 59-year-old female with type 2 diabetes mellitus, and an 8-year and 9-month-old female who was first discovered to harbor a mutation in the xanthine dehydrogenase gene using whole-exome sequencing, but had a normal molybdenum cofactor sulfurase gene. Hydantoin-5-propionate was detected in the first and third patients but not in the second, suggesting that the first and second patients had type I and II xanthinuria, respectively.

Conclusion: Gas chromatography/mass spectrometry-based metabolomics can be used for undiagnosed patients with xanthinuria, identification of the type of xanthinuria without allopurinol loading, and the quick functional evaluation of mutations in the xanthinuria-related genes.

Key words: hydantoin-5-propionate, hypouricemia, metabolomics, urolithiasis, xanthinuria.

Keynote message

Three patients with xanthinuria were identified using GC/MS-based urine metabolomics. Prior to metabolomics, one patient was discovered to harbor a mutation in the xanthine dehydrogenase gene using whole-exome sequencing, necessitating the evaluation of mutation effect. Typing of type I or type II xanthinuria will be enabled by evaluating hydantoin-5-propionate.

Introduction

Xanthinuria is an inborn error of metabolism in the final stage of purine catabolism.[1] Xanthinuria type I (MIM 278300) is caused by the deficiency of XDH.[2] Xanthinuria type II (MIM 603592) is caused by the mutation of the *MOCOS*, resulting in the combined deficiency of XDH and AO.[3] Molybdenum cofactor deficiency (MIM 252150), often called xanthinuria type III, results from mutations in *MOCS1*, *MOCS2*, and the *GEPH* and causes triple deficiency of XDH, AO, and sulfite oxidase, frequently resulting in progressive neurological damage and early childhood death.[4]

Detecting xanthinuria is important to prevent urolithiasis and drug-induced toxicity. Correct exclusion of xanthinuria is necessary for early diagnosis of hereditary renal hypouricemia or EIAKIs, which have a higher estimated incidence.[5,6]

We conducted high-risk screening for 130 inborn errors of metabolism, using GC/MS-based metabolomics.[7] Herein, we report three cases of xanthinuria.

Presentation of cases

Case 1

A 72-year-old male presented with macroscopic hematuria and urinary retention. Flexible cystourethroscopy revealed stenosis at the bulbar urethra. The narrowed lumen was expanded, and an indwelling bladder catheter was installed. One month after first presentation, the patient was admitted to undergo cystostomy because of repeated catheter problems. Abdominal X-ray and cystoscopic examination revealed no stones, but bladder diverticulum. After 1.5 months, a bladder stone measuring 22.8 × 8.8 mm was visualized using cystoscopy. The stone was removed via an endourologic approach and was confirmed to be struvite. There were no signs indicating urinary stones, except for painless gross hematuria. Preoperative renal function tests, including estimated glomerular filtration rate (107.21 mL/min/1.73 m^2) were normal. Spot urine and serum were examined using metabolomics to identify the etiology of stone formation.

Case 2

A 55-year-old female with a 10-year history of type 2 diabetes mellitus had a low urate level (0.04 mg/dL), and the FEUA was 6.1%. She also had sudden sensorineural hearing loss and dyslipidemia. Notably, her sibling had hypouricemia, and her parents are cousins. The patient had no history of urolithiasis or EIAKI. At 59 years of age, her spot urine and serum were examined using metabolomics to identify the etiology of hypouricemia.

Case 3

A healthy 1-year-old girl developed frequent bloody stools 2 days after the onset of vomiting and diarrhea. Fluid therapy improved her dehydration. Five days after onset of symptoms, she became pale, and laboratory examination revealed hemolytic anemia with a hemoglobin level of 5.0 g/dL and thrombocytopenia of 36 × 10^3/μL. The serum creatinine level more than doubled from the basal value of 0.22 mg/dL to 0.55 mg/dL, indicating AKI. Her serum uric acid level was only 0.2 mg/dL. The patient was diagnosed with thrombotic microangiopathy, probably HUS, following diarrhea and bloody stools. No Shiga toxin-producing pathogenic *E. coli* was detected in the stool. The patient's serum complement levels were not decreased, and there was no clear evidence of atypical HUS and systemic lupus erythematosus as the cause of thrombotic microangiopathy.

Moreover, the levels of a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13 (ADAMTS-13) had not decreased, ruling out thrombotic thrombocytopenic purpura. Thorough physical examination and imaging studies showed no evidence of urinary tract obstruction. After supportive care including fluid replacement and red blood cell transfusions, HUS resolved without plasma therapy or dialysis.

There was no known consanguinity in the family. Even while developing AKI, the serum urate level was low (0.2 mg/dL); after recovery from AKI, it dropped to 0.1–0.2 mg/dL, with a 2.1% FEUA. Later, the patient had sensorineural hearing loss in her right ear. At the age of 7 years, she experienced severe left hemi-abdominal pain with gross hematuria. Ultrasound examination did not show any urinary stones. Hematuria and pain resolved spontaneously over 2 days, strongly suggesting renal stones.

Whole-exome sequencing revealed compound heterozygosity of c.3847C > T (p. Arg1283*) and c.1242G > C (p. Glu414Asp) in the XDH; xanthinuria type I. c.3847C > T, registered as rs751921838 in the SNP database (https://www.ncbi.nlm.nih.gov/snp/rs751921838#publications), causes nonsense mutation, a novel mutation of XDH.[8] c.1242G > C is a splice-site mutation not registered in the SNP database. c.1242G > C was predicted as benign by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.bii.a-star.edu.sg/), and as disease-causing by Mutation Taster (http://www.mutationtaster.org/index.html). These results appeared compatible with her hypouricemia with low FEUA level, but were inconclusive. Her *MOCOS* status was normal, and xanthinuria type II was ruled out. Her urine was examined using metabolomics to evaluate the function of this genetic variant.

All three patients were identified to have the typical metabolic profile of xanthinuria (Fig. 1; Table 1). Their data were compared with those of a sibling with xanthinuria type III[9] that causes triple deficiency of XDH, AO, and sulfite oxidase.

Discussion

XDH and AO are drug-metabolizing enzymes, whose absence causes drug-induced toxicity.[10] The two types of xanthinuria are clinically indistinguishable; however, types I and II can be differentiated using an allopurinol-loading test. Peretz *et al*. subtyped xanthinuria into types I and II (i.e., combined XDH/AO deficiency) by assessing biomarkers specific to AO, including hydantoin-5-propionate, using LC/MS-based untargeted metabolomics of urine samples.[11] We detected normal urine levels of hydantoin-5-propionate in Cases 1 and 3 but not in Case 2 or xanthinuria type III. Therefore, Case 1, but not Case 2, had xanthinuria type I. Hydantoin-5-propionate evaluation using GC/MS would facilitate rapid differentiation of type I and type II/type III.

Prior to metabolomics, Case 3 was discovered to harbor a mutation in *XDH*, but not in *MOCOS*, via whole-exome sequencing. Metabolomics can be used to assess the biochemical or clinical significance of a mutation,[12] and thus, complements whole-exome sequencing. However, failure to detect a pathogenic mutation using conventional genetic analysis does not exclude a disease, particularly where an abnormal metabolic phenotype has been detected.[13]

Patients with xanthinuria type I and II can be asymptomatic or symptomatic.[14] In a Czech population, nine cases (four asymptomatic) of hereditary xanthinuria were detected, suggesting that this is a common disorder of purine metabolism.[15] Over 50% of the reported cases were symptomatic; therefore, early diagnosis may prevent disease progression, severe complications, and end-stage renal disease.

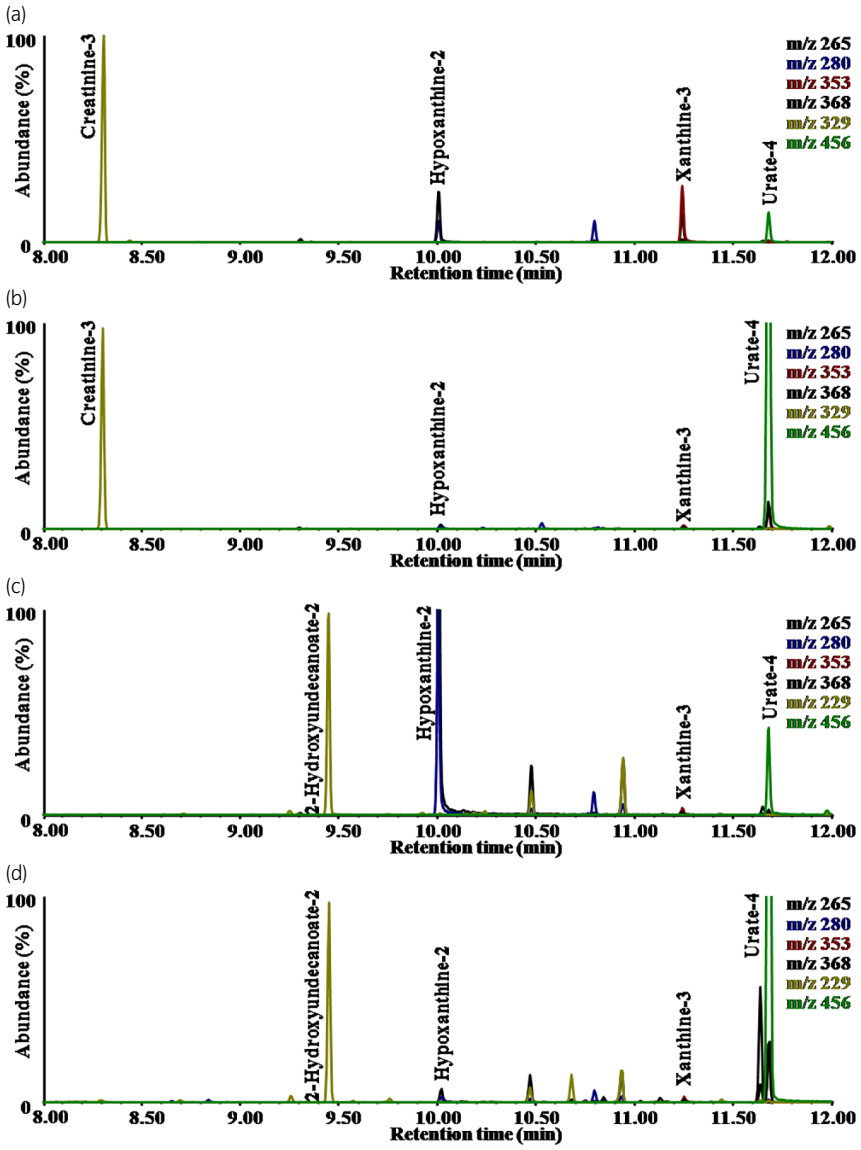

Fig. 1 Mass chromatograms of the trimethylsilyl derivatives of hypoxanthine, xanthine, urate, and creatinine from urine and serum. Sample preparation and GC/MS measurement were the same as those described previously.[7] Metabolites in the urine were evaluated as creatinine-based.[7] Metabolites in the serum were evaluated using 2-hydroxyundecanoate, 25 μmol of which was added per 0.1 mL of serum. (a) Urine from Case 1, (b) urine from an age-matched control, (c) serum from Case 1, and (d) serum from an age-matched control.

Table 1 *Z*-scores of the biomarkers, hypoxanthine, xanthine, urate, and hydantoin-5-propionate[†]

	Case 1		Case 2		Case 3	MOCS1[‡]	MOCS1[‡]
Target	Urine	Serum	Urine	Serum	Urine	Urine	Urine
Hypoxanthine	4.0	7.8	4.3	2.0	5.0	5.9	4.3
Xanthine	8.1	3.7	10.0	5.0	6.0	3.5	4.5
Urate	−5.6	0.9	−12.0	−3.5	−8.0	−5.5	−14.5
Σ[§]	12.1	11.5	14.3	7.0	11.0	9.4	8.8
Hydantoin-5-propionate[¶]	0.9	—	−3.7	—	1.3	<−4	<−4

Urine was more sensitive than serum as an indicator of urate production inability or hypouricemia. †*Z*-score was calculated after $\log_{10}$-transformation, as described.[12] In the metabolomics studies performed, the quantitative values of biomarkers are not obtained. Instead, creatinine-based relative values are obtained. *Z*-score (*n*) indicates the degree of deviation of a patient's biomarkers from control values. $n = (V_p - V_{mean}) / SD$, V_p: measured value in a patient; V_{mean}: mean of measured values in control group. V_p and each value in control groups are all $\log_{10}$ transformed. For example, in case 2, *Z*-score (4.3) of hypoxanthine was derived from V_p, $\log_{10}(V_p)$, V_{mean}, SD, $\log_{10}(V_p) - V_{mean}$, the values of which were 2.11, 0.324, −0.557, 0.204, and 0.881, respectively. ‡MOCS1 denotes the molybdenum cofactor synthesis 1 gene responsible for xanthinuria type III. Data are from siblings diagnosed with mutations in MOCS1.[9] §Sum of *Z*-scores of hypoxanthine and xanthine. ¶The AO-specific biomarker. SD, standard deviation.

We demonstrated the usefulness of urine metabolomics for patients with urolithiasis or hypouricemia owing to xanthinuria. Patients who form stones owing to inborn errors of metabolism may be a minority among all patients with urolithiasis. However, we propose that, for patients with stone-forming conditions whose diagnosis has been delayed for months, years, or more, and those with hypouricemia, GC/MS-based urine metabolomics can help rapidly identify those having inborn errors of metabolism.

Acknowledgments

Part of this study was supported by Grants-in-Aid (20ek0109301s4003) from the Japan Agency for Medical Research and Development.

Author contributions

Tomiko Kuhara: Conceptualization; formal analysis; methodology; resources; validation; writing – original draft; writing – review and editing. Masahiro Tetsuo: Conceptualization; investigation; resources; writing – original draft; writing – review and editing. Morimasa Ohse: Formal analysis; investigation; visualization. Toshihiko Shirakawa: Investigation; resources; writing – original draft. Yumiko Nakashima: Investigation; resources; writing – original draft. Koh-ichiro Yoshiura: Investigation; resources; writing – original draft. Nagaaki Tanaka: Investigation; resources; writing – original draft. Tadashi Taya: Conceptualization; investigation; resources; supervision.

Conflict of interest

The authors declare no conflict of interest.

Approval of the research protocol by an Institutional Reviewer Board

All procedures in this study that involved human participants were performed in accordance with the ethical standards of the Institutional Review Board and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Written informed consent was obtained from all individual participants included in the study.

Registry and the Registration No. of the study/trial

Not applicable.

Funding information

None.

References

1 Simmonds HA, Reiter S, Nishino T. Hereditary xanthinuria. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *The Metabolic and Molecular Bases of Inherited Diseases* McGraw-Hill, New York, 1995; 1781–97.
2 Ichida K, Amaya Y, Kamatani N, Nishino T, Hosoya T, Sakai O. Identification of two mutations in human xanthine dehydrogenase gene responsible for classical type I xanthinuria. *J. Clin. Invest.* 1997; **99**: 2391–7.
3 Ichida K, Matsumura T, Sakuma R, Hosoya T, Nishino T. Mutation of human molybdenum cofactor sulfurase gene is responsible for classical xanthinuria type II. *Biochem. Biophys. Res. Commun.* 2001; **282**: 1194–200.
4 Reiss J, Johnson JL. Mutations in the molybdenum cofactor biosynthetic genes MOCS1, MOCS2, and GEPH. *Hum. Mutat.* 2003; **21**: 569–76.
5 Iguchi A, Sato T, Yamazaki M *et al*. A case of xanthinuria type I with a novel mutation in xanthine dehydrogenase. *CEN Case Rep.* 2016; **5**: 158–62.
6 Wakasugi M, Kazama JJ, Narita I *et al*. Association between hypouricemia and reduced kidney function: a cross-sectional population-based study in Japan. *Am. J. Nephrol.* 2015; **41**: 138–46.
7 Matsumoto I, Kuhara T. A new chemical diagnostic method for inborn errors of metabolism by mass spectrometry-rapid, practical, and simultaneous urinary metabolites analysis. *Mass Spectrom. Rev.* 1996; **15**: 43–57.
8 Tanaka K, Kanazawa I, Yamasaki H, Hasegawa H, Ichida K, Sugimoto T. Xanthinuria type I with a novel mutation of xanthine dehydrogenase. *Am. J. Med. Sci.* 2015; **350**: 155–6.
9 Higuchi R, Sugimoto T, Tamura A *et al*. Early features in neuroimaging of two siblings with molybdenum cofactor deficiency. *Pediatrics* 2014; **133**: e267–71.
10 Stiburkova B, Pavelcova K, Petru L, Krijt J. Thiopurine-induced toxicity is associated with dysfunction variant of the human molybdenum cofactor sulfurase gene (xanthinuria type II). *Toxicol. Appl. Pharmacol.* 2018; **353**: 102–8.
11 Peretz H, Watson DG, Blackburn G *et al*. Urine metabolomics reveals novel physiologic functions of human aldehyde oxidase and provides biomarkers for typing xanthinuria. *Metabolomics* 2012; **8**: 951–9.
12 Kuhara T. Gas chromatographic-mass spectrometric urinary metabolome analysis to study mutations of inborn errors of metabolism. *Mass Spectrom. Rev.* 2005; **24**: 814–27.
13 Coene KLM, Kluijtmans LAJ, van der Heeft E. Next-generation metabolic screening: targeted and untargeted metabolomics for the diagnosis of inborn errors of metabolism in individual patients. *J. Inherit. Metab. Dis.* 2018; **41**: 337–53.
14 Kubihal S, Goyal A, Singla R, Khadgawat R. Urolithiasis due to hereditary xanthinuria type II: a long-term follow-up report. *Indian Pediatr.* 2020; **57**: 468–9.
15 Sebesta I, Stiburkova B, Krijt J. Hereditary xanthinuria is not so rare disorder of purine metabolism. *Nucleosides Nucleotides Nucleic Acids* 2018; **37**: 324–8.