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Metabolomics analysis of blood identifies potential biomarkers and possible treatment targets for nocturia

Satoru Kira, Takahiko Mitsui, Tatsuya Miyamoto, Tatsuya Ihara, Hiroshi Nakagomi, Yuka Hashimoto, Hajime Takamatsu, Masayuki Tanahashi, Masahiro Takeda, Norifumi Sawada and Masayuki Takeda

Abstract

Background: Our aim was to investigate the association between serum metabolites and nocturia.

Methods: A total of 66 males aged 65–80 years were enrolled in this study and stratified according to micturition behavior, which was characterized in terms of the 24 h frequency volume chart (FVC) for 3 consecutive days, the International Prostate Symptom Score (IPSS), and quality-of-life score. The nocturia group included participants with any total IPSS and $\geqslant$1.5 micturitions/night as the mean of 3 nights, while the control group included participants with total IPSS $<$ 8 and $<$1.5 micturitions/night. We conducted a comprehensive capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) study of plasma metabolites. Between-group comparisons of metabolite levels employed the Welch *t* test. The relationship between nocturia and metabolite profiles was determined using multivariable logistic regression analysis.

Results: Of 66 participants, 45 were included in the nocturia group and 21 in the control group. There were no differences in background factors between the two groups. FVC analysis revealed that urine production during night-time, as well as micturition frequency during daytime and night-time were significantly higher in the nocturia group. CE-TOFMS identified eight metabolites whose plasma levels differed between the two groups. Multivariate analysis indicated that increased levels of lauric acid and imidazolelactic acid, as well as decreased levels of thiaproline and glycerol, contribute to the etiology of nocturia in aged men.

Conclusions: Our findings suggest that abnormal serum levels of metabolites in several pathways play a role in the pathogenesis of nocturia in aged men.

Keywords: aged men, metabolomics analysis, nocturia

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Correspondence to:
Satoru Kira
Department of Urology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Shimokato 1110, Chuo, Yamanashi 409-3898, Japan
skira@yamanashi.ac.jp

Takahiko Mitsui
Tatsuya Miyamoto
Tatsuya Ihara
Hiroshi Nakagomi
Norifumi Sawada
Masayuki Takeda
Department of Urology, University of Yamanashi, Chuo, Japan

Yuka Hashimoto
Hajime Takamatsu
Masayuki Tanahashi
Masahiro Takeda
Pharmacology Research Labs, Astellas Pharma Inc., Tsukuba, Japan

Introduction

Nocturia is a common lower urinary tract symptom (LUTS) with a negative influence on quality of life.[1] According to the frequency volume chart (FVC), nocturia can be categorized as global polyuria, nocturnal polyuria (NP), reduced bladder capacity, and sleep disorder.[2] Lifestyle-related comorbidities, such as diabetes mellitus, chronic kidney disease, cardiac insufficiency, and metabolic syndrome (Mets), can also play an important role in the pathophysiology of nocturia.[3] Thus, currently available therapeutic interventions for nocturia were developed to target such factors. However, as nocturia reflects the concerted action of several factors and specific conditions in each individual, it remains challenging to achieve treatment success while ensuring safety using a single therapeutic approach in all patients with nocturia. Thus, in order to develop new treatment approaches, drugs, or preventive strategies, it is necessary to elucidate the details of the pathophysiology of nocturia.

The metabolomic approach aims to provide insight into the specific metabolic phenotype associated with a disease.[4] Capillary electrophoresis time-of-flight mass spectrometry (CE -TOFMS)-based metabolomic analysis provides important advantages, including high resolution, ability to analyze the metabolite profiles of various organisms, and ability to quantify virtually all changes in the levels of low-molecular-weight compounds in a sample.[5–8] For this reason, CE-TOFMS-based metabolomic analysis has been successfully used in the detection of biomarkers for psychiatric disorders and lower urinary tract disorders, including interstitial cystitis and male LUTS.[4,9,10] For example, a recent report described the association between LUTS and amino acid profiles in the plasma.[11] However, no study has reported the implementation of a CE-TOFMS pipeline for differential serum metabolomics analysis of patients with nocturia and controls. In this study, we performed a CE-TOFMS-based metabolomics analysis to elucidate the association between serum metabolites and nocturia in a sample of aged men with and without nocturia.

Materials and methods

Study design

This study was performed in accordance with the Declaration of Helsinki and its latest amendments, following approval by the Regional Ethics Committee of our university. Prior to initiating data collection, all participants provided oral and written informed consent.

Participants

This study enrolled 66 men aged 65–80 years, recruited from the outpatient clinic of our hospital. For the assessment of LUTS, all participants filled out the FVC for three consecutive days, the International Prostate Symptom Score (IPSS) questionnaire, and a quality-of-life questionnaire. The nocturnal polyuria index (NPi) was calculated as urine production during night-time divided by 24 h urine production. NP was defined as NPi $>$ 0.33.[12] The nocturia group was defined as participants with any total IPSS and $\geqslant$1.5 micturitions per night as the mean of three nights from data of FVC, while the control group was defined as participants with total IPSS $<$ 8 and $<$1.5 micturitions per night. We excluded individuals with cancer, bladder pain syndrome, urinary tract infection, polyuria (40 ml/kg/24 h), NPi $>$ 70%, residual urine $>$100 ml, or serious systemic complications such as central nervous system disorders, chronic kidney disease, and heart failure. Detailed information about the participants and their FVC data was provided previously.[13]

Serum sampling and preparation

Blood samples of 3 ml from fasting participants were acquired at the outpatient clinic of our department between 0800 and 0900 and stored at −80°C. A plasma volume of 50 µl was added to 450 µl of methanol containing internal standards and mixed. Then, 500 µl of chloroform and 200 µl of MilliQ water were added, and the resulting solution was mixed again. After centrifugation at 2300*g* for 5 min, an aliquot of 400 µl from the aqueous layer was sampled and filtered using a 5 kDa membrane filter, followed by drying under reduced pressure. The residue was reconstituted with 25 µl MilliQ water for CE-TOFMS analysis (all reagents from Human Metabolome Technologies Inc., Tsuruoka, Japan).

CE-TOFMS analysis

CE-TOFMS was carried out using an Agilent CE Capillary Electrophoresis System equipped with an Agilent 6210 TOF mass spectrometer, Agilent 1100 isocratic HPLC pump, Agilent G1603A CE-MS adapter kit, and Agilent G1607A CE-ESI-MS sprayer kit (Agilent Technologies, Waldbronn, Germany). The systems were controlled using the Agilent G2201AA ChemStation software, version B.03.01, for CE (Agilent Technologies). The metabolites were separated using a fused-silica capillary (internal diameter, 50 µm; total length, 80 cm) with a commercial running and rinse buffer for electrophoresis (solution ID: H3301-1001 for cation analysis and I3302-1023 for anion analysis; Human Metabolome Technologies) as the electrolyte. The sample was injected at a pressure of 50 mbar for 10 sec (approximately 10 nl) for cation analysis and for 25 s (approximately 25 nl) for anion analysis. The spectrometer was scanned over an m/z range from 50 to 1000.

Peaks were extracted using the automatic integration software MasterHands version 2.16.0.15 (Keio University, Tsuruoka, Japan) in order to obtain peak information, including *m*/*z*, CE

migration time, and peak area. Signal peaks corresponding to isotopomers, adduct ions, and other product ions of known metabolites were excluded, and the remaining peaks corresponding to putative metabolites were annotated based on the corresponding reference migration/retention times and m/z values included in the Human Metabolite Technologies metabolite database. The tolerance range for peak annotation was configured at ± 0.5 min for migration time and ± 10 ppm for m/z. In addition, the ratios of peak areas of metabolites to internal standards were used in the statistical analyses.

Statistical analyses

Participant characteristics and FVC data were analyzed using the unpaired Welch t test. Categorical variables were evaluated using Fisher's exact test. Metabolite profiles were compared between the nocturia group and the control group using the Welch t test as a nonadjusted analysis. To investigate the relationship between metabolite profiles and nocturia, we performed multivariable logistic regression analysis, and the results were expressed as odds ratio with 95% confidence interval. The following factors were used for covariate adjustment: age (continuous), body mass index (continuous), 24 h urine production (continuous), use of drugs for treatment of LUTS (yes/no), and presence of hypertension, diabetes, or hyperlipidemia (yes/no) as metabolic comorbidities. Relationships with $p < 0.05$ were considered statistically significant. Statistical analyses were performed using SPSS version 22 (IBM Corp., Armonk, NY, USA).

Results

Of the 66 men included in the study, 45 were allocated to the nocturia group and 21 to the control group. No differences were found between the two groups with regards to age, body mass index, or prevalence of a specific lifestyle-related disease, but the number of participants receiving anticholinergic drugs or having at least one metabolic comorbidity (any of the three lifestyle-related diseases considered here) was significantly higher in the nocturia group (Table 1).

Among the FVC parameters, the micturition frequency during daytime and night-time, urine production during night-time, NPi, and number of NPs were significantly higher in the nocturia group. In contrast, the voided volume per micturition during daytime and night-time was significantly smaller in the nocturia group. No significant between-group differences were found in urine production during daytime or 24 h urine production (Table 2).

Among the 218 (126 cationic and 92 anionic) metabolites surveyed, 106 annotated metabolites were selected for further statistical analyses after excluding artificial chemicals and biological metabolites with detection rate lower than 20% among the 66 subjects. The nonpaired Welch t test revealed that eight metabolites identified using CE-TOFMS had plasma levels that differed significantly between the nocturia and control groups (Table 3).

Multivariable logistic regression analysis revealed that increased levels of lauric acid and imidazolelactic acid, as well as decreased levels of thiaproline and glycerol were significantly associated with nocturia (Table 4).

Discussion

This is the first study to employ CE-TOFMS-based metabolomic analysis for identifying biomarkers of nocturia in the serum of aged men. Specifically, CE-TOFMS revealed eight serum metabolites associated with nocturia, while multivariable logistic regression analysis suggested that increased levels of lauric acid and imidazolelactic acid, as well as decreased levels of thiaproline and glycerol are significantly associated with nocturia. The present findings suggest that abnormal levels of metabolites involved in several pathways may play a role in the pathogenesis of nocturia.

Lauric acid is a saturated fatty acid, indicated by the results of our logistic regression analysis, as a serum biomarker of nocturia in aged men. Lauric acid, which represents the main fatty acid found in coconut oil, has a favorable effect on total serum cholesterol levels, mainly by increasing the levels of high-density lipoprotein (HDL) cholesterol.[14] Increased serum levels of HDL cholesterol are strongly associated with reduced incidence of atherosclerosis. Thus, lauric acid (or coconut oil) is expected to reduce the risk of cardiovascular disease (CVD) by modulating serum cholesterol levels. Of the various Mets components, dyslipidemia is believed to be associated with nocturia or LUTS. Indeed, a previous epidemiological survey indicated that hypertriglyceridemia is significantly associated with nocturia or

Table 1. Characteristics of the participants enrolled in this study ($n = 66$).

Characteristic	Control ($n = 21$)	Nocturia ($n = 45$)	p value
	Mean ± SD	Mean ± SD	
Age, years	70.5 ± 4.0	72.3 ± 4.0	0.1119
Body mass index, kg/m^2	22.9 ± 2.4	23.0 ± 3.0	0.8802
Lifestyle-related diseases	*n*	*n*	
Hypertension	2	14	0.0697
Diabetes mellitus	1	3	>0.99
Hyperlipidemia	0	5	0.1691
Any of the above	2	17	0.0209
Drug for LUTS	*n*	*n*	
α1-receptor antagonist	12	36	0.0753
5α-reductase inhibitor	5	8	0.7406
Anticholinergic drug	0	8	0.0478
β3-receptor agonist	2	9	0.4802
PDE5 inhibitor	1	1	0.5385

A, alpha; β, beta; SD, standard deviation; LUTS, lower urinary tract symptoms; PDE, phosphodiesterase.

Table 2. Frequency volume chart data.

Parameter	Control ($n = 21$)	Nocturia ($n = 45$)	p value
	Mean ± SD	Mean ± SD	
Micturition frequency during daytime	6.28 ± 2.2	7.96 ± 2.3	0.0055
Micturition frequency during night-time	0.69 ± 0.5	2.47 ± 0.7	<0.0001
Voided volume per micturition during daytime, ml	208 ± 83	158 ± 63	0.0084
Voided volume per micturition during night-time, ml	307 ± 132	204 ± 57	<0.0001
24 h urine production, ml	1530 ± 480	1715 ± 413	0.11
Urine production during daytime, ml	1055 ± 425	1022 ± 337	0.74
Urine production during night-time, ml	475 ± 150	694 ± 177	<0.0001
NPi	0.325 ± 0.1	0.41 ± 0.09	0.0005
Nocturnal polyuria patients, *n*	8	35	0.002

SD, standard deviation; NPi, nocturnal polyuria index.

LUTS.[15,16] Based on these observations, it is considered that a positive effect of lauric acid in CVD may translate into a favorable effect in nocturia. However, the results of this study indicated that lauric acid might have a harmful effect in nocturia. Furthermore, whether or not lauric acid

Table 3. Metabolites showing significant differences in serum levels between the nocturia group and control group.

Compound	Ratio	Nonadjusted *p* value
Lauric acid	1.10	<0.001
Thiaproline	0.76	0.002
5-methoxyindoleacetic acid	1.28	0.002
Imidazolelactic acid	1.20	0.013
Glycerol	0.86	0.017
5-hydroxylysine	1.23	0.02
NG, NG-dimethyl-L-arginine	1.10	0.039
Betaine	1.16	0.042

The *p* values refer to the differences between the two groups. The ratio was obtained as the value noted for the nocturia group, divided by the value noted for the control group.

Table 4. Logistic regression analysis of potential serum biomarkers of nocturia.

Compound	Odds ratio	95% CI	*p* value
Lauric acid	34.364	3.251, 363.2	0.003
Thiaproline	0.003	0, 0.192	0.006
5-methoxyindoleacetic acid	5.78	0.991, 33.694	0.051
Imidazolelactic acid	14.297	1.029, 198.633	0.048
Glycerol	0.728	0.534, 0.992	0.045
5-hydroxylysine	1.819	0.92, 3.6	0.086
NG, NG-dimethyl-L-arginine	2.075	0.318, 13.521	0.445
Betaine	3.441	1.002, 11.812	0.050

CI, confidence interval.

truly reduces CVD risk remains uncertain.[14,17] Thus, further study is needed to clarify the relationship between lauric acid and nocturia.

In this study, the levels of thiaproline, which is a condensate product of formaldehyde reacted with cysteine, were inversely associated with nocturia. Formaldehyde is a common reactive carbonyl product generated from both endogenous oxidative stress and several exogenous sources.[18] Thiaproline has been investigated for its pharmacological properties and suggested to have several biological activities including anti-inflammatory effect.[19] Thus, our results suggest that in aged men with nocturia, a decrease in thiaproline levels may reflect elevation or relapse of inflammation. A previous community-based survey reported that the serum levels of the inflammatory marker C-reactive protein were significantly associated with LUTS and nocturia.[20] Taking into account these previous observations and our present findings, we speculate that inflammation caused by decreased thiaproline levels in the serum may result in nocturia.

We found that serum glycerol concentrations were significantly lower in the nocturia group than in the control group. Glycerol is produced

during lipolysis, which involves hydrolysis of triglycerides stored in the adipocytes of adipose tissue. As participants included in the nocturia group had lower levels of glycerol, we may infer that lipolysis was downregulated in these individuals. Excess accumulation of triglycerides in the adipose tissue induces obesity and results in Mets. Considering that in this study, men with nocturia also had a higher prevalence of metabolic comorbidities, we hypothesize that Mets played a key role in nocturia due to downregulation of lipolysis. However, it has been suggested that basal lipolysis (i.e. lipolysis in the absence of stimulatory factors, which include several hormones) is closely associated with obesity and insulin resistance.[21] Since we could not discriminate between metabolites produced by hormone-dependent lipolysis and those produced by basal lipolysis, further research is warranted to clarify the relationship between lipolysis and nocturia.

In our present study, linear regression analysis indicated that increased levels of imidazolelactic acid are associated with nocturia in aged men. Imidazolelactic acid is derived from histidine, which is a precursor to histamine. Although a previous CE-TOFMS-based study suggested an association between schizophrenia and imidazolelactic acid, the details of this association remain unknown.[22] Further research is warranted to clarify the association between imidazolelactic acid and nocturia.

The FVC data in this study indicate that NP was the most common pathophysiologic manifestation of nocturia, which is consistent with previous observations.[23] NP is associated with urine overproduction at night, which may be influenced by lifestyle-related diseases associated with Mets.[23,24] On the other hand, Mets consists of several components known to represent CVD risk factors, including obesity, high blood pressure, high blood-sugar levels, and dyslipidemia. A community-based survey suggested an association between Mets and urological symptoms, including nocturia.[15] However, the exact factors underlying the association between Mets and nocturia remain unclear. In this study, the prevalence of NP and metabolic comorbidities representing lifestyle-related diseases was higher in the nocturia group than in the control group. Taking into account the main results of this study, namely the association between nocturia and changes in plasma levels of lauric acid, imidazolelactic acid, thiaproline, and glycerol, we hypothesize that pathological features of regulatory pathways involved in dyslipidemia, including abnormal lipolysis and inflammation, underlie both nocturia and Mets.

This study has several limitations. First, the sample was small and included only aged men. Thus, we could not examine our results in the context of different etiologies of nocturia, which include NP, reduced bladder capacity, and mixed disorders. In addition, this study had a cross-sectional design. Furthermore, the etiology of nocturia has multiple factors, and it is possible that our results do not reflect all such factors. Nonetheless, our present findings reveal pathways that may serve as therapeutic targets or at least lead to the discovery of suitable targets for the treatment of nocturia. To provide a detailed characterization of the serum metabolite profile in nocturia, further longitudinal studies with larger sample size covering female and male patients with nocturia are warranted.

Conclusions

In this CE-TOFMS-based metabolomic study, we identified several serum metabolites significantly associated with nocturia. Our findings suggest that abnormal levels of metabolites involved in several pathways contribute to the etiology of nocturia in aged men, indicating potential therapeutic targets for the development of new treatments for nocturia. Future research is needed to verify these results on a larger scale.

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Conflict of interest statement

Yuka Hashimoto, Hajime Takamatsu, Masayuki Tanahashi, and Masahiro Takeda are employed by Astellas Pharma Inc. The other authors declare that there is no conflict of interest.

ORCID iD

Satoru Kira https://orcid.org/0000-0001-5282-3832

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