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"*Nocturomics*": transition to omics-driven biomarkers, a systematic review and future prospects

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Keywords: Nocturomics, nocturia, biomarker, metabolomics, microbiome

Wordcount:

Abstract: 255 words

Manuscript: 3590 words

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Abstract:

Objective: To systematically review studies that investigated different biomarkers of nocturia, including omics-driven biomarkers or "Nocturomics".

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bju.15975

Materials and Methods: PubMed®, Scopus®, and Embase® were searched systematically in May 2022 for research papers on biomarkers in physiological fluids and tissues from patients with nocturia. A distinction was made between biomarkers or candidates discovered by omics techniques, referred to as omics-driven biomarkers, and classical biomarkers, measured by standard laboratory techniques and mostly thought from pathophysiological hypothesis.

Results: Thirteen studies with 18,881 patients in total were included, 8 of which focused on classical biomarkers, including the atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-reactive protein (CRP), aldosterone, and melatonin. Five were "Nocturomics", including one that assessed the microbiome and identified 27 fecal and 8 urinary bacteria correlated with nocturia and 4 studies that identified candidate metabolomic biomarkers, including fatty acid metabolites, serotonin, glycerol, lauric acid, thiaproline, and imidazolelactic acid among others. To date, no biomarker is recommended in clinical practice. Nocturomics are in an embryonic phase of conception but are developing at a high speed. Although candidate biomarkers are being identified, none of them are yet validated on a large sample size although some preclinical studies have shown a probable role of fatty acid metabolites as a possible biomarker of circadian rhythm and chronotherapy.

Conclusion: Further research is needed to validate biomarkers for nocturia within the framework of a diagnostic and therapeutic precision medicine perspective. We hope this study provides a summary of the current biomarker discoveries associated with nocturia and details future prospects for omics-driven biomarkers.

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Introduction

Nocturia, defined by the International Continence Society as being awakened by the need to urinate during the primary sleep period, represents a symptom associated with pathologies that may or may not be directly related to the urinary tract [1]. It has a significant societal and economic impact, altering quality of life with loss of work productivity, emotional distress, behavioral changes, and increased risk of falls [2].

Several pathologies may be intertwined in the etiopathogenesis of nocturia including obstructive sleep apnea, congestive heart failure, diabetes, peripheral edema, and excessive fluid intake prior to nighttime [3]. On the other hand, these conditions have different characteristics, unique molecular profiles, and responses to therapeutic agents; therefore, not all patients respond to the same treatment protocols in the same way, as existing treatment options are determined according to the underlying cause [4]. Based on the complex physiopathology, several trials have been carried out in the search of biomarkers of nocturia, from blood or urine samples to brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP), and melatonin. However, there has been no applicable clinical translation. Hence, while adherence to treatment and outcomes are complex and closely related, non-adherence to treatment of nocturia is common [4]. Therefore, there is an urgent need to detect accurate biomarkers that will facilitate the diagnosis, prognosis, and management of nocturia and to develop personalized medicine modalities.

From a precision medicine perspective, the logic behind biomarker discovery is quite complex. Theoretically, if we had sufficient information on the molecular pathophysiology of the conditions related to nocturia, it would be possible to define biomarkers. In practice, nocturia is more complex, and a multi-omics approach is necessary. Omics-driven biomarkers have a

fundamentally different approach to data gathering than classical biomarkers. Classical biomarkers use deductive reasoning by looking at specific biomarkers to test their sensitivity, specificity, and clinical translation while omics-driven biomarkers use inductive reasoning by using an entire dataset of biomarkers to identify among other things new potential pathophysiological hypotheses for nocturia [5]. Over the past two decades, technological and computational discoveries have revolutionized the ability to obtain high-throughput data at almost any molecular level through multi-omics. They gather information from several fields, such as microbiomics, which is concerned with the community of microbes and their genes in a patient; and metabolomics, which analyzes all the low-molecular-weight metabolites such as amino acids [6]. Interest in the microbiome is gaining momentum; a recent review has demonstrated its close association with urological cancers [7] and recently a gut microbiotic signature was identified among kidney stone formers [8]. Another study showed a close link between microbial colonization and lower urinary tract symptoms (LUTS) [9] where women with significant bacteriuria were more likely to have nocturia, urgency, bladder pain, urgency incontinence, and nocturnal enuresis. Although there are limited studies, a recent systematic review has demonstrated that the urine microbiome of overactive bladder (OAB) patients is more predisposed to alteration from the gut or vaginal influences than in healthy controls [10]. These developments have contributed to the understanding of the underlying molecular mechanisms of LUTS. Although in the early stage of assessment, some of these proposed markers may distinguish the characteristics of nocturia and become future diagnostic, prognostic, and therapeutic targets.

This work reviews the results obtained so far in biomarker discovery of different omics levels, especially microbiomics and metabolomics. We present a comprehensive review of what is known

about multi-omics biomarkers and their role in the pathogenesis of nocturia while discussing limitations and providing future prospects of this innovative field.

Materials and Methods

The systematic review of studies of different markers in nocturia was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) in Supplementary Table 1. The protocol was registered on the International Prospective Register of Systematic Reviews database (CRD42022345598).

Search Strategy

In May 2022, two authors (GBK and SG) designed and executed literature searches in PubMed, EMBASE, and SCOPUS for studies that included nocturia and biomarkers. The search string included keywords such as "nocturia" and "biomarker", "-omics", "genomics", "metabolomics", "microbiomics", "proteomics", "transcriptomics", or "lipidomics". Initial screening was performed independently by two investigators (GBK and SG) based on the titles and abstracts of the article. Reasons for exclusions were noted and potentially relevant studies were subjected to a full-text review. Data summaries confirmed the relevance of the papers and disagreements were resolved via consensus with the co-authors.

Inclusion and exclusion criteria

Two authors (GBK and SG) independently screened articles for English, French, and Dutch studies that met the inclusion criteria. Studies were included if they assessed biomarkers of nocturia including omics-driven biomarkers. Only clinical studies involving adult patients over the age of 18 were included. We accepted studies if biomarkers of nocturia were analyzed in a nocturic

subgroup of patients among other lower urinary tract symptoms (LUTS). We excluded papers that studied pediatric populations, animal studies, and studies that did not analyze a subgroup of nocturic patients. Systematic reviews, case reports, and studies with insufficient data were excluded.

Data Summary

Two authors (GBK and SG) created a data summary table to record the following information: first author's last name, sample size, sample characteristics (nocturia vs. controls), definition of nocturia, -omics measured, and all biomarker outcomes analyzed. All discrepancies regarding data extraction were resolved by consensus with the committee of authors.

Separation of Omics-Driven Biomarker Studies

After data summary, studies were classified into those that were searching for specific biomarkers and those that looked at the entire data collection of biomarkers of which a subset were found to be significant. The latter group used the novel omics-driven approach and thus, the outcomes measured were classified as omics-driven biomarkers.

Risk of bias assessment

Quality and risk of bias were evaluated by 2 authors (GBK and SG) for the selected articles based on the Critical Appraisal Skills Program (CASP) checklist for "classical biomarkers" case-control and observational studies. Studies were considered adequate if 8 to 10 items of the checklist were valid and partially adequate if 5 to 7 items were valid [7]. The "omics-driven biomarkers" studies were assessed based on the QUADOMICS checklist, designed specifically for omics-driven studies with 9 elements presented in Supplementary Table 2 [11].

Results

Characteristics of studies

Our initial search identified 258 publications. After the elimination of duplicates and sorting through titles and abstracts, 24 studies were selected for full-text review. Most studies were excluded because they didn't focus on nocturia. Based on selection criteria, 13 studies were identified, comprising 18,881 patients for the systematic review. The detailed retrieval process is shown in figure 1.

Eight studies were identified as classical biomarker studies in Table 1 [12–19]. Five of these eight studies identified increased BNP/ANP [12,13,16,17,20] , two studies identified increased C-reactive protein (CRP)[14,15], two studies showed decreased aldosterone[12,17], and two studies showed decreased melatonin[17,19]. Additionally, studies also showed increased noradrenaline and dopamine[17], and decreased arginine vasopressin (AVP) and spot urine sodium excretion[12,20]. These changes were all associated with the presence and severity of nocturia.

For omics-driven biomarkers, although we searched for metabolomics, microbiomics, proteomics, transcriptomics, and lipidomics, only articles discussing metabolomics or the microbiome were included. Thus, five studies were categorized as omics-driven biomarker studies in Table 2[21–25]. Of these five studies, two evaluated the urinary metabolome [21,24], with identification of the involvement of serotonin, 3-hydroxyproprionic acid, and 3-indoleacetonitrile, and two others evaluated the serum metabolome [23,25] with identification of 9 metabolites of fatty acids, glycerol, thiaproline, lauric acid and imidazolelactic acid. These were all associated with nocturia.

One study assessed the microbiome[26] and identified 27 fecal and 8 urinary bacteria correlated with nocturia. In general, the omics-driven biomarker studies were more recently published.

Risk of bias assessment

Based on the CASP checklist, Supplementary Table 4 shows that 3 of the 8 included studies were classified as only partially adequate due to small sample sizes and potential confounding factors in the design/analysis [12,14,16], while 5 were adequate [13,15,17,19,20]. The QUADOMICS evaluation concerning the omics-driven biomarkers is presented as a Supplementary Table 3. The biases are more or less similar between the 5 included omics studies that described the pre-analytical phase well, with criteria for patient selection and sample sampling. However, in no case was the statistical method detailed enough to know if there was quality control through cross-validation.

Discussion

This systematic review is the first in the literature to describe the different biomarkers studied for nocturia, which we termed "Nocturomics". We explored how metabolomics and the microbiome have possible clinical translations and correlations to an already established pathophysiological base. We separated the classical biomarker studies from the omics-driven biomarker studies, based on the approach and technique to elucidate the changing field of biomarker identification in nocturia. The evolution of omics-driven biomarkers allows for the identification of new biomarkers, which may elucidate the pathophysiology of nocturia and aid in the personalization of future therapies.

Classical Biomarkers

Common identified biomarkers were increased BNP/ANP (5 studies) [12,13,16–18], increased CRP (2 studies) [14,15], decreased aldosterone (2 studies) [12,17], and decreased melatonin (2 studies) [17,19]. These studies were attempting to support theories suggested previously about the association of nocturia with these biomarkers.

The association between increased BNP/ANP and nocturia has been noted for many years. Natriuretic peptides regulate the circadian rhythm, dilate blood vessels, and cause the kidney to excrete more salt and water by suppressing renin and aldosterone release[27,28]. Studies from the 1990s have suggested a relationship between increased natriuretic peptides and nocturia[29]. Since then, multiple studies, which have been included in our systematic analysis, have shown similar findings. ANP levels are known to increase in the elderly [12]. There are many candidate pathophysiological mechanisms linking increased natriuretic peptides to nocturia, which include obstructive sleep apnea (OSA), sodium imbalance, and abnormal circadian blood pressure patterns [12,16]. Higher BNP levels are also associated with OSA, a cause of nocturia [16]. Restriction of sodium intake has been reported to reduce BNP and nocturia, thus excess sodium intake may be involved in this association [30,31]. In the elderly, as renal blood flow decreases due to aging, nocturnal edema increases the circulating blood volume due to dysfunctions in reabsorption, thereby increasing ANP/BNP and urine production[17]. Increased nocturnal natriuretic peptides cause increased nocturnal blood pressure, which is associated with nocturia[18,19].

Additionally, research has found that increased CRP levels, a marker of chronic inflammation, were positively associated with nocturia[14]. Chronic inflammation occurs due to the aging process and produces free radicals, which are associated with LUTS such as nocturia [32,33]. Chronic inflammation is also a cause of bladder storage failure, which has been associated with

OAB and nocturia[34]. Furthermore, the treatment of LUTS with antimuscarinic agents has shown a significant decrease in elevated serum CRP levels[35].

In addition, aldosterone is a key molecule in water homeostasis and is released due to increased plasma osmolality or severe hypovolemia, resulting in increased water reabsorption. Low aldosterone levels at night, an age-related alteration in aldosterone secretion, especially the loss of circadian oscillation of aldosterone plasma levels, may contribute to nocturia in the elderly [36].

Finally, melatonin was decreased in two studies. Melatonin is a hormone released exclusively at night and has been shown to synchronize the circadian rhythms and improve the onset, duration, and quality of sleep by being centrally involved in sleep regulation[28,37]. The proposed mechanisms of the association between reduced melatonin and nocturia include sleep disorders and increased functional bladder capacity[19]. As melatonin is a regulator of nocturnal sleep, decreases in this biomarker would cause sleep disturbances[17]. Poor sleep quality also causes nocturnal polyuria in healthy adults[19]. Additionally, in a previous study, exogenous melatonin was seen to increase bladder capacity in rats[38]. So, decreased melatonin may decrease bladder capacity, resulting in nocturia.

In addition to BNP/ANP, aldosterone, and CRP, some included studies also saw an increase in both daytime noradrenaline and dopamine as well as a decrease in both nocturnal AVP and spot urine sodium excretion. The prevalence of nocturia in persons with untreated hypertension is 68% in men and women and 49% in men whose hypertension was medically treated but uncontrolled[17,39]. Increased noradrenaline and dopamine causes hypertension, which increases renal arterial resistance, decreases renal blood flow, and can lead to insufficient daytime urine production[17]. When catecholamines decrease at night, renal arterial resistance decreases and

renal blood flow increases, which allows urine production to increase to excrete water retained in various tissues throughout the body during the daytime [17]. Also, AVP is a major regulator of water excretion by the kidneys and works with aldosterone to increase water reabsorption. Decreased AVP in elderly patients may be due to excessive fluid intake and water retention from increased circulating catecholamines and hypertension [17]. Finally, the inverse association between spot urine sodium excretion and nocturnal urination frequency may support an involvement of salt sensitivity in nocturia [20]. Although total urinary sodium excretion per day represents daily sodium intake, daytime sodium excretion of nonsensitive individuals is relatively higher than that of salt-sensitive individuals, and salt-sensitive individuals excrete more sodium during the night than nonsensitive individuals [20].

Overall, the associations between altered levels of natriuretic peptides, CRP, aldosterone, and melatonin to nocturia have been supported by different studies. Although associations occur, no diagnostic tests or treatment regimen utilizes these biomarkers in nocturic patients [40]. Since these biomarkers have been studied for decades but are still of little clinical significance, other, as of yet unidentified, biomarkers may also be at play in fully explaining the pathophysiology of nocturia. Thus, we also systematically reviewed omics-driven biomarkers to elucidate a different method for future biomarker discovery.

Omics-Driven Biomarkers

Multi-omics is the molecular characterization and quantification of biological molecules in various subfields of molecular biology that can be explored in serum, urine, feces, and tissues, leading to the generation of large quantitative omics datasets that will subsequently be exploited by complex automated learning statistical methods [41]. As there is now an infinite number of "omics" terms

derived from different types of biomolecules in the cells of the human body, we will limit our approach to the omics that have recent application in nocturia research.

Metabolomics is the measurement of certain intracellular metabolites to provide the concentration of small molecules in biological fluids to create a "fingerprint" unique to the individual's physiological and pathological conditions. Several analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS) as well as capillary-electrophoresis mass-spectrometry (CE-MS) have been widely used in metabolomic studies [42]. Four studies were used to identify potential metabolomic biomarkers of nocturia in older men [21,23–25]. One study using LC-MS [24] identified 3 metabolites correlated with nocturia out of 291 metabolites measured; increased serotonin, decreased 3-hydroxypropionic acid, metabolite of beta-alanine, and decreased 3-indoleacetonitrile, metabolite of tryptophan. These metabolites underscore the established clinical correlation between depression, metabolic syndrome, and nocturia [43]. The role of serotonin is well known in circadian rhythm disorders [44] and nocturia is itself considered as a form of circadian dysfunction [45]. Moreover, major depression is associated with an increased risk of nocturia, which has been accounted for by a central or peripheral serotonergic effect and on sleep disturbances in depression [46]. In the same vein, reduced 3-hydroxypropionic acid, a metabolite of beta-alanine, underlines the correlation between depression and nocturia, since beta-alanine itself has been shown to be involved in stress management, and diets rich in beta-alanine have been shown to have a favorable preclinical antidepressant and anxiolytic effects in mice [47]. As altered levels of these metabolites may play a role in the association between nocturia and a reduction in mental well-being, physicians should consider routine assessment of mental health, conceptualization and inventory of mental health burden, in a multidisciplinary approach to the treatment of nocturia patients. As for the metabolic

syndrome, the intestinal microbiome seems to be more and more involved in its physiopathology, among other things, via the reduced indole derivatives produced via tryptophan metabolism dysregulation [49]. A high body mass index (BMI) also seems to correlate with a urinary metabolic profile of increased 2-hydroxyisobutyrate and isoleucine and lower concentrations of hippurate, phenylacetylglutamine, tyrosine, and threonine demonstrated by Bray et al. who analyzed by NMR 176 women with LUTS [21]. However, in this same study, KODAMA analysis showed a correlation between BMI, parity, overactive bladder syndrome, frequency, straining and bladder storage but not nocturia, a difference that may be explained by both the definition of nocturia changing between one study and another, and the omics sequencing technique used (Table 2). Increased fatty acid metabolites were also identified in the results of studies that looked for serum metabolomic biomarkers of nocturia [23,25]. This discovery led to preclinical research evaluating the circadian rhythm of fatty acid metabolism and their effect on voiding in mice [50], which showed that increased serum levels of fatty acid metabolites during sleep from circadian rhythm disturbances can cause nocturia and that fatty acid metabolic pathways were involved in the regulation of clock genes. Based on circadian rhythm disorders and recent findings on chronotherapy [51], these results may have two future dimensions, the first pathophysiological hypothesis of the involvement of fatty acid metabolites in nocturia and the second clinical with a possibility of monitoring circadian rhythm via metabolomic markers of fatty acids in serum. This lends to therapeutic interventions individualized by metabolomic markers.

Although the research into the microbiome is gaining momentum in concepts of overactive bladder pathogenesis [10], its role in nocturia is not yet broadly studied. It was studied in fecal and urinary samples in a population of 30 males presenting with LUTS [22] which determined that 27 fecal and 8 urinary bacteria were correlated with nocturia using 16s ribosomal RNA sequencing.

Although this is a single study of 30 patients, it is noteworthy to parallel these findings with a preclinical study where a rodent model that was subjected to four weeks of daily sleep fragmentation showed both an increased abundance of the Lachnospiraceae and Ruminococcaceae families, and a decrease in Lactobacillaceae, all of which were correlated with nocturia [52]. These findings can suggest a pathophysiological circle where the bladder-gut-brain triangle links the gut microbiome to sleep and circadian misalignment, and then to the brain center controlling the biological clock and the bladder-brain network. Gut dysbiosis can alter the gut metabolome which could contribute to chronic inflammation, positive energy balance, and endocrine disruption, which in turn can promote the development of metabolic syndrome that is itself correlated with nocturia [51,53].

The findings of our review have several limitations. By using stringent criteria, many studies may have been excluded. Additionally, despite conducting comprehensive searches, articles that were published in journals not indexed in databases used in our analysis could have been missed. Also, the definition of nocturia changes often. There is still disagreement about whether ≥ 1 void/night is sufficient for nocturia diagnosis or if there must be ≥ 2 voids/night. Specifically for omics-driven biomarkers, the techniques vary widely and there is no standard method to sequence omics nor a standardized statistical approach in identifying the candidate biomarkers. Additionally, three omics-driven studies were done by the same expert group, with the same sample of nocturia patients, but with different sequencing techniques and statistical analysis. Since these studies make up most omics-driven biomarker studies, any errors in sample size or technique will carry over to our study. This also means a less diverse group of people are represented in our review. Additionally, the lack of studies on the sensitivity and specificity of all biomarkers and their association to nocturia makes it difficult to measure the clinical significance of these findings.

Despite these limitations, our review provides a systematic analysis which associates several biomarkers with nocturia and provides an avenue for future research. The process of biomarker development comprises four main steps: discovery, analytical validation, evaluation of clinical utility, and clinical use [6] summarized in figure 2. Thus, since "*nocturomics*" studies are only in the primary stage, the next step is a standardization of techniques to allow the use of reproducible sequencing methods combined with robust statistical analysis methods integrating expert knowledge-driven approach to identify a place for new biomarkers in the identification of the pathophysiological causes of nocturia. Validation studies with a more representative sample of age-matched controls are warranted, the purpose being an integrative precision medicine approach. Once the standardization of techniques and analytical validation have occurred, hypotheses about nocturomics can be generated for clinical use, followed by animal experimentation. Further exploration can include the use of genomics, transcriptomics, and proteomics to predict the occurrence and/or severity of nocturia as there was no present literature using these -omics techniques. For example, the development of molecular ecological signatures of health may allow accurate identification of dysbiosis and allow at-risk communities to be screened for proactive interventions. Information about the role of specific -omics in the development of LUTS may allow for a more personalized treatment plan. Additionally, a combination of big data and machine learning with novel omics-driven techniques can be used to collect and integrate a vast amount of information about nocturia in an efficient way. Research about deep-learning and machine-learning models for predicting prognosis in cancer patients is already being conducted using multi-omics data[54]. Finally, the evaluation of mental health status and nocturia severity with potential biomarkers for both conditions warrant future research.

Conclusion

Although recent evidence contributes to our current understanding of "*nocturomics*", its clinical translation is still seminal. In any case, existing evidence suggests that specific taxa and their modified or microbiome-derived metabolites are correlated with nocturia, which may itself be a component of metabolic derangement and circadian dysregulation, opening the possibility for further research to validate biomarkers not only of nocturia but also of circadian rhythm disorders, facilitating improved application of chronotherapeutic approaches.

Declaration of Interest

This work received no funding. We certify that all conflicts of interest, including specific financial interests, relationships, and affiliations relevant to the subject matter or materials discussed in the manuscript are the following: JW is a consultant for Ferring and the Institute for Bladder and Prostate Research. KE reports grants for Ferring and from Medtronic outside the submitted work and is a minor shareholder without salary from P2Solutions (smart textile applications). The other authors have no conflicts of interest (including everything stated under the ICMJE Disclosure Form) to declare.

Legends

Figure 1: Prisma flowchart

Figure 2: The workflow of a biomarker discovery in the setting of nocturia. OTU = Operational Taxonomic Unit, CRP = C-reactive peptide, ANP = Atrial Natriuretic Peptide, BNP = B-type natriuretic peptide, rRNA = ribosomal ribonucleic acid, NMR = nuclear magnetic resonance, LC-MS = liquid chromatography-mass spectrometry, CE-MS = capillary electrophoresis mass-spectrometry.

Table 1: Blood and urine biomarkers of nocturia

Table 2 : Omics-driven biomarkers. OTUs= Operational Taxonomic Unit, rRNA= ribosomal ribonucleic acid, IPSS = International Prostate Symptom Score, ICIQ-FLUTS= International Consultation on Incontinence Questionnaire – female Lower Urinary Tract Symptoms.

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Table 1. Blood and urine biomarkers of nocturia

Study	Sample Size	Sample Characteristics (nocturia vs. controls)	Definition of Nocturia	Assayed Materials	Outcomes Measured
Carter, 1999[12]	34	18 vs. 16	F/V chart	Blood and urine samples	• ↑ ANP • ↓ AVP • ↓ aldosterone
Hung, 2014[14]	853	–	IPSS	Blood sample	• ↑ CRP
Kupelian, 2009[15]	3752	–	AUA-SI, nocturnal void ≥ 1	Blood sample	• ↑ CRP
Obayashi, 2014[19]	861 community elderly	261 vs. 600	Urination diary, nocturnal void ≥ 2	ELISA on urine	• ↓ 6-sulfatoxymelatonin
Okumura, 2021[16]	1096	328 vs. 768	Urination diary, nocturnal void ≥ 2	Fasting blood sample	• ↑ BNP
Sugaya, 2008[17]	180	60 vs. 120	Nocturnal void ≥ 2	Daytime and nighttime blood sample analysis	• ↑ daytime and nighttime ANP and BNP • ↑ nighttime plasma noradrenaline • ↑ daytime and nighttime dopamine • ↓ daytime and nighttime aldosterone • ↓ nighttime melatonin
Tabara, 2019[20]	5683 community residents	–	Sleep diary, IPSS	Blood specimen and spot urine samples	• ↑ BNP • ↓ spot urine sodium excretion
Yoshimura, 2012[13]	5980	745 vs. 5235	IPSS and OABSS, nocturnal void ≥ 2	Fasting blood sample	• ↑ BNP

Table 2: Omics-driven biomarkers. OTUs= Operational Taxonomic Unit, rRNA= ribosomal ribonucleic acid, IPSS = International Prostate Symptom Score, ICIQ-FLUTS= International Consultation on Incontinence Questionnaire – female Lower Urinary Tract Symptoms.

Study	Sample Size	Sample Characteristics (nocturia vs. controls)	Definition of Nocturia	Omics Estimation	Outcomes Measured
Bray, 2017[21]	214 women	176 vs. 36	ICIQ-FLUTS ≥ 1	Metabolomics Urine samples Nuclear magnetic resonance (NMR)	• Identification of 4 clusters of urinary metabolomic profiles without any correlation with nocturia.
Kira, 2018[23]	66 men	45 vs. 21	Average of 3 nights < 1.5 micturitions/night	Metabolomics Serum samples Liquid-chromatography mass-spectrometry (LC-MS)	• ↑ palmitoylethanolamide • ↑ 4-hydroxydocosahexaenoic acid • ↑ 20-hydroxydocosahexaenoic acid • ↑ 9-hydroxyoctadecadienoic acid • ↑ 13-hydroxyoctadecadienoic acid • ↑ arachidonoylethanolamide • ↑ eicosapentaenoic acid • ↑ 12-hydroxy-eicosatetraenoic acid • ↑ arachidonic acid
Kira, 2019[24]	66 men	45 vs. 21	Average of 3 nights < 1.5 micturitions/night	Metabolomics urinary samples LC-MS	• ↑ serotonin • ↓ 3-hydroxypropionic acid • ↓ 3-indoleacetonitrile
Kira, 2019[25]	66 men	45 vs. 21	Average of 3 nights < 1.5 micturitions/night	Metabolomics Plasma samples Capillary electrophoresis time-of-flight mass spectrometry	• ↑ lauric acid • ↑ imidazolelactic acid • ↓ thiaproline • ↓ glycerol
Holland, 2020[22]	30 men	–	IPSS	Microbiome	<u>Fecal OTUs:</u>

				Urinary and fecal samples 16s rRNA	• Bacteroidaceae, Bacteroides; • Bacteroidetes, Prolixibacter; • Clostridiales Peptoniphilus; • Enterobacteriaceae, Klebsiella; • Fusobacteriaceae, Fusobacterium; • Lachnospiraceae, Blautia; • Lachnospiraceae, Clostridium_XlVa; • Lachnospiraceae, Coprococcus; • Lachnospiraceae, Dorea; • Methanopyraceae, Methanopyrus; • Prevotellaceae, Prevotella; • Porphyromonadaceae, Odoribacter; • Rhodospirillaceae, Dongia; • Rikenellaceae, Alistipes; • Ruminococcaceae, Subdoligranulum; • Streptococcaceae, Streptococcus; • Succinivibrionaceae, Succinivibrio; • Sutterellaceae, Sutterella; • Veillonellaceae, Allisonella; • Veillonellaceae, Dialister <u>Urinary OTUs:</u> • Actinomycetaceae, Actinomyces; • Aerococcaceae, Facklamia; • Corynebacteriaceae, Corynebacterium; • Lachnospiraceae, Dorea; • Lachnospiraceae, Roseburia; • Ruminococcaceae, Faecalibacterium; • Ruminococcaceae, Gemmiger

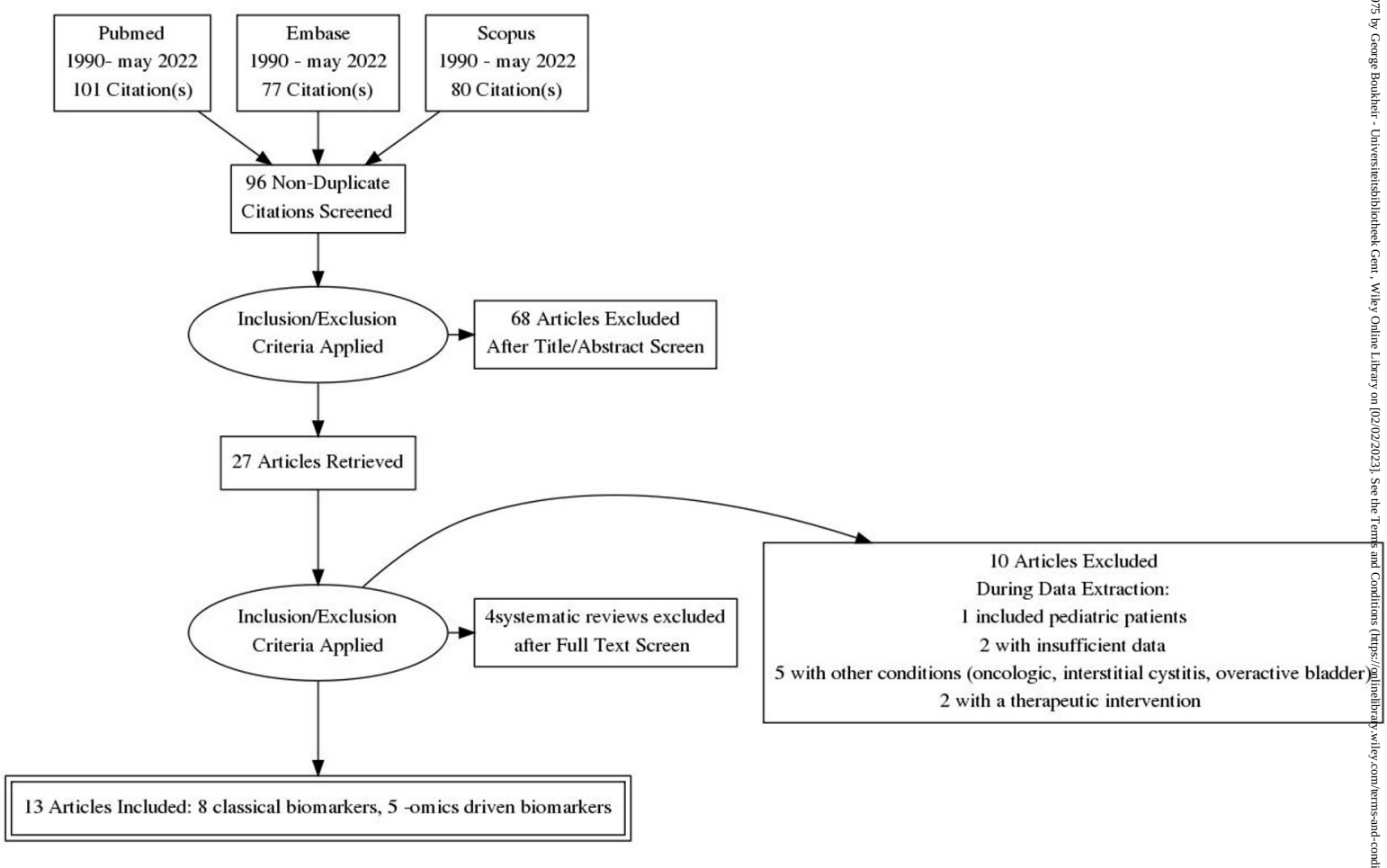

Figure 1 Prisma flowchart.

BJU_15975_BJU Figure 1.jpg

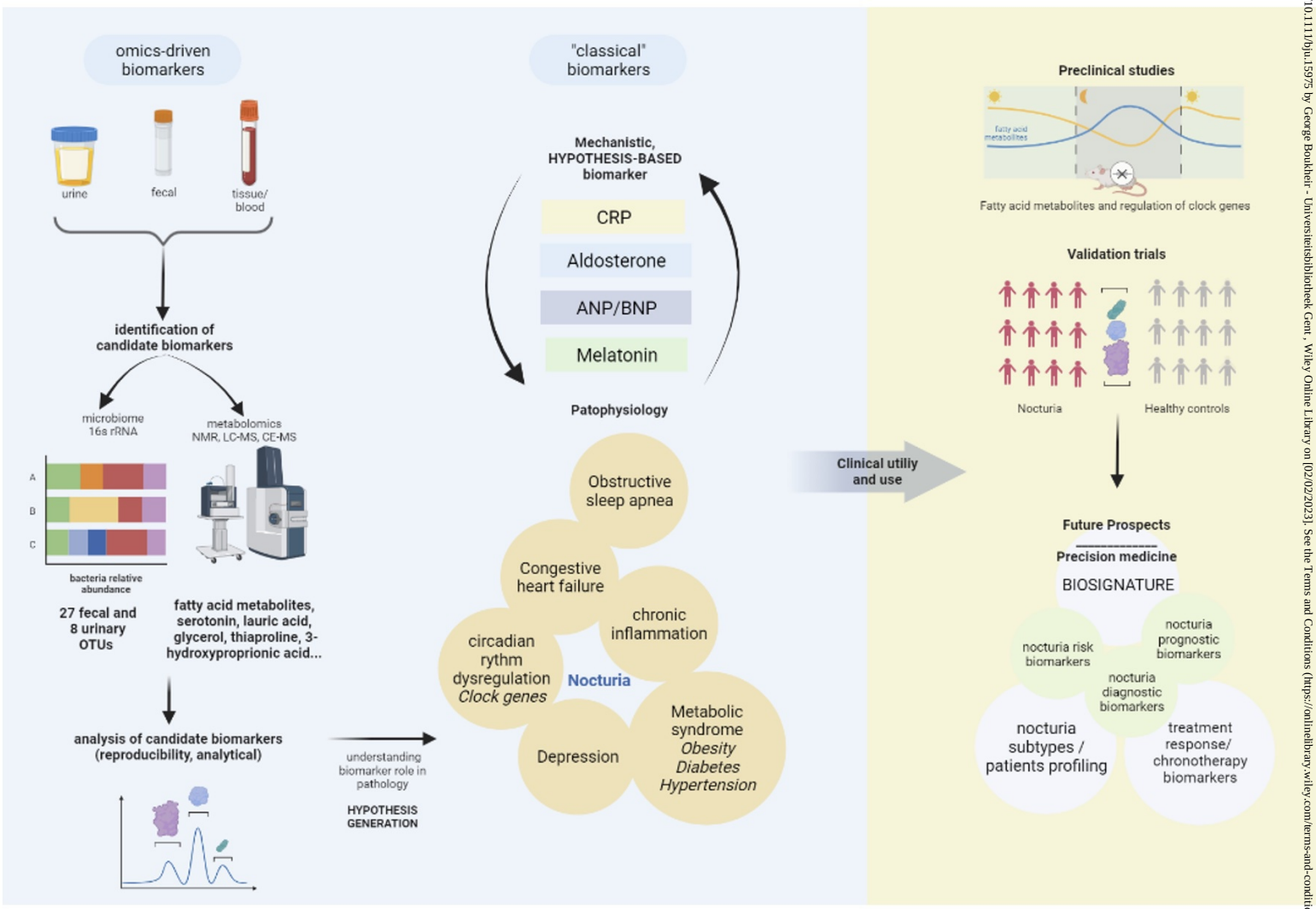

Figure 2 The workflow of a biomarker discovery in the setting of nocturia. OTU = Operational Taxonomic Unit, CRP = C-reactive peptide, ANP = Atrial Natriuretic Peptide, BNP = B-type natriuretic peptide, rRNA = ribosomal ribonucleic acid, NMR = nuclear magnetic resonance, LC-MS = liquid chromatography-mass spectrometry, CE-MS = capillary electrophoresis mass-spectrometry.

BJU_15975_BJU Figure 2.jpg