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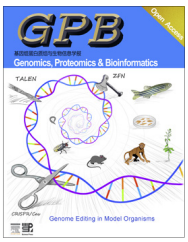

REVIEW

Urinary Biomarkers of Brain Diseases

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Abstract **Biomarkers** are the measurable changes associated with a physiological or pathophysiological process. Unlike blood, **urine** is not subject to homeostatic mechanisms. Therefore, greater fluctuations could occur in **urine** than in blood, better reflecting the changes in human body. The roadmap of **urine** biomarker era was proposed. Although **urine** analysis has been attempted for clinical diagnosis, and **urine** has been monitored during the progression of many diseases, particularly urinary system diseases, whether **urine** can reflect brain disease status remains uncertain. As some **biomarkers** of **brain diseases** can be detected in the body fluids such as cerebrospinal fluid and blood, there is a possibility that **urine** also contain **biomarkers** of **brain diseases**. This review summarizes the clues of **brain diseases** reflected in the **urine** proteome and metabolome.

Introduction

The use of effective biomarkers has great significance for the prediction, diagnosis, monitoring, treatment, and prognosis of many diseases. Urine is not subject to homeostatic mechanisms and accommodate many changes that may reflect status of the body, such as pregnancy, aging, and daily rhythms [1]. These changes may be used as promising biomarkers [2]. Therefore the roadmap of urine indicator as the future of biomarker applications was proposed [3]. Currently, most studies on urinary biomarkers have focused on kidney diseases due to the close relationship between the kidneys and urine [4,5]. The lack of attention to urinary biomarkers in other diseases, like brain diseases, may be due to the fact that anatomically, the brain and urine are not closely related and there exists the filtering effect of the blood–brain barrier and the kidneys. Most brain disease studies have focused on cerebrospinal fluid (CSF) and blood [6,7], in which changes caused by brain diseases may be attenuated by homeostatic mechanisms. However, the potential presence of brain disease biomarkers in urine is largely neglected, and there are only a few urinary biomarker studies on brain diseases. Although the brain and urine are

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distantly apart and a direct relationship is hard to establish with current scientific knowledge, we cannot rule out the possibility that changes present in the brain are somehow reflected in urine. Here, we summarize urinary proteomics and metabolomics applications for the study on brain diseases, which may be useful for researchers interested in exploring the field.

Omics technology including proteomics and metabolomics has garnered a lot of attention recently in the biomarker field thanks to its high-throughput and high-sensitivity capacities. Urine has been commonly examined in omics fields. Proteins in the urine mainly come from blood by filtration and the kidneys/urinary tracts by secretion. It was previously believed that urine contains very few proteins. However, a recent study indicated that the content of urinary proteins from normal subjects is 0–0.8 g/l [8]. Nonetheless, urine contains fewer high abundance proteins than blood, which makes the identification of urinary proteins relatively easy since no high abundance suppression exists. Six thousand proteins can be identified in urine, indicating the complexity of the urine proteome and highlighting the availability of the urine proteome for the study of biomarkers (personal communication). Currently, urinary proteomics is mainly used to search for biomarkers of kidney diseases, such as IgA nephropathy [9], renal cell carcinoma [10], and kidney transplant [11]. However, urinary proteomics can also be used to study a variety of diseases, such as sleep apnea [12], eclampsia [13], and cardiovascular diseases [14].

Through urinary metabolomics, small molecule metabolites in urine with molecular weights less than 1000 Da can be comprehensively analyzed. Metabolites in urine may reflect state of the body to some extent and serve as informative biomarkers for some diseases. Urinary metabolomics has been widely used in the studies of various diseases, including diabetic nephropathy [15], acute kidney injury [16], chronic heart failure [17], liver cancer [18], and breast cancer [19].

The main purpose of the biomarker studies is to identify specific and stable biomarkers for a particular disease. Because many diseases are heterogeneous, finding a single biomarker relative to one disease is difficult. Thus, a panel of proteins or metabolites identified by omics technology is considered feasible and would be desirable biomarkers of the disease. In addition, the search for brain disease biomarkers in urine may pose challenges because their changes in the urine are sensitive and can be affected by many factors transiently. However, only stable changes that are strongly associated with disease can be effective biomarkers. Such changes can only be considered specific if they are independent of impact of transient factors. In the current review, we summarized previous studies searching brain diseases biomarkers in urine by proteomics and metabolomics.

Neuropsychiatric disorders

Major depressive disorder

Major depressive disorder (MDD) is a severe neuropsychiatric disorder that is characterized by disordered mood, cognition, neurovegetative functions, and mental activity [20]. Clinically, the diagnosis of MDD is mainly based on a subjective symptom assessment, while no objective and effective measurements are available so far [21]. Therefore, biomarkers that can enhance traditional symptom-based assessments and predict treatment response are greatly needed in clinical settings.

In order to search biomarkers of MDD, urine of 42 first-episode drug-naïve MDD subjects and 28 normal subjects was analyzed using a peptidomics method, which mainly targeted the small polypeptides. Five peptides (m/z 8307.22, 3222.17, 4640.35, 5072.14, and 1196.47) were selected from the 29 putative urinary peptides for establishing a candidate classification model. This model showed good diagnostic performance for MDD with a 90.5% sensitivity (38/42), a 92.9% specificity (26/28), and a 91.4% accuracy (64/70). Four of these five peptides have been identified to correspond to four known proteins, including serum albumin (m/z 1196.47), alpha-1-microglobulin/bikunin precursor (AMBP, m/z 3222.17), heparan sulfate proteoglycan (HSPG, m/z 4640.35), and apolipoprotein A-I (APOA1) (m/z 5072.14) [22]. Among them, altered expression of APOA1 has previously been correlated with several other psychiatric disorders [23,24].

In addition, urinary metabolomics was also used for MDD biomarker identification. Urinary metabolomes of a training set (82 first-episode, drug-naïve MDD subjects and 82 normal subjects) were measured using nuclear magnetic resonance (NMR) [25]. Metabolites associated with tricarboxylic acid (TCA) cycle, intestinal microflora metabolism, and tryptophan-nicotinic acid pathway were altered in the MDD patients' urine. A receiver-operating characteristic (ROC) curve analysis was performed to evaluate the validity of these possible biomarkers. A biomarker classification model consisting of formate, malonate, *N*-methylnicotinamide, m-hydroxyphenylacetate, and alanine showed the highest predictive power for MDD, in which the area under the ROC curve (AUC) of this classification model was 0.81 in the training samples and 0.89 in the test samples. This specific classification model also performed well in a test set despite of a lack of age matching. In a follow-up study, urine from the same MDD and control subjects were analyzed by gas chromatography-mass spectrometry (GC–MS) [26]. Six metabolites (sorbitol, uric acid, azelaic acid, hippuric acid, quinolinic acid, and tyrosine) were selected from 23 differential metabolites and considered promising biomarkers for MDD diagnosis (0.905 of AUC in training samples and 0.837 of AUC in test samples). A composite model add *N*-methylnicotinamide, which was identified in a previous study [25], to the six aforementioned metabolites performed even better (AUC: 0.909 in training samples and 0.917 in test samples) compared to the current model with six metabolites only in distinguishing MDD patients from controls [25]. Metabolic pathway analysis showed disturbances in peripheral glucose metabolism, perturbed kynurenine pathway metabolism, disturbances in the tyrosine-phenylalanine pathway, and increased oxidative stress in MDD patients. These two studies identified some candidate urinary biomarkers with the potential for diagnosing MDD in the future clinical setting and demonstrated the power of the metabolomics-based method for searching MDD biomarkers. However, large samples from a heterogeneous population are needed to validate the applicability and sensitivity of these candidate diagnostic models. The correlation between these differential metabolites and MDD must also be further investigated to understand the pathological mechanisms and therapeutic targets.