Urine Proteomics for Prediction of Escitalopram Efficacy

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Abstract

Abstract Objective: Escitalopram requires approximately 12 weeks to demonstrate efficacy in the treatment of depression. We sought to explore the prediction of drug efficacy before medication administration using a urinary proteome approach.

Methods: We selected urine samples from 10 good responders, 10 poor responders, and 9 healthy controls. To highlight each patient' s personalized response to the drug, we employed a one-to-many comparison approach, comparing the pre-treatment urinary proteome of an individual depressed patient with that of a group of healthy controls (n=9) to identify biological pathways.

Results: Among the biological pathways specific to good responders, 6 patients (60%) were enriched in the glycine synthesis pathway. Additionally, the biological pathways identified in both patient groups included previously reported depression-associated HIF-1 , STAT3, serotonin receptors, and ceramide pathways, suggesting that these pathways may also represent potential antidepressant targets.

Conclusion: The Mayo Clinic previously reported that glycine dehydrogenase is associated with escitalopram treatment efficacy in depressed patients through single nucleotide polymorphism (SNP) analysis. The results of this genetic study are in complete concordance with our urinary proteomics study. The urinary proteome has the potential to predict escitalopram efficacy in a subset of patients before treatment.

Full Text

Urinary Proteomics Predicts the Efficacy of Escitalopram

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Abstract

Objective: Escitalopram treatment for depression requires 12 weeks to take effect. We aimed to use urinary proteomics to explore the prediction of drug efficacy before administration.

Methods: We selected urine samples from 10 patients with good treatment response, 10 with poor response, and 9 healthy individuals. To highlight the individualized response of each patient, we employed a one-to-many comparison approach, comparing the urinary proteome of a depressed patient with a group of healthy individuals (n=9) before medication to identify biological pathways.

Results: In the biological pathways unique to the good-response group, 6 patients (60%) were enriched in the glycine synthesis pathway. Additionally, the biological pathways of both patient groups included previously reported depression-related HIF1 , STAT3, serotonin receptor, and ceramide pathways, suggesting these may also be potential antidepressant targets.

Conclusion: A previous Mayo Clinic study identified glycine dehydrogenase as associated with escitalopram efficacy in depressed patients through SNP analysis. This genetic study was completely consistent with our urinary proteomics results, suggesting that the glycine synthesis pathway is more likely associated with escitalopram treatment outcomes. Urinary proteomics has potential to predict escitalopram efficacy in some patients before treatment.

Keywords: Depression, medication efficacy, glycine pathway, escitalopram, urinary proteome

Introduction

Major depressive disorder is a debilitating mental illness affecting 12.3% of the global population and impacting 15% of individuals during their lifetime. Depression is a complex heterogeneous disorder with multifactorial etiology involving biological, genetic, and social factors that remain incompletely understood. Critically, only 50% of patients respond effectively to antidepressant treatment. While major depression generally requires pharmacological intervention, the variable efficacy of antidepressants represents a persistent clinical challenge. Short-term effects may be adaptive, but long-term therapeutic outcomes are often inadequately evaluated.

Because depression diagnosis relies on behavioral abnormalities, treatment remains symptomatic rather than targeting specific pathophysiological mechanisms. Providing effective personalized medication guidance for each patient is an urgent clinical need. While psychopharmacological innovation is essential, identifying new molecular targets remains difficult, primarily due to limited understanding of antidepressant mechanisms. Clinically, robust evidence is needed to select optimal treatment strategies for individual patients.

Urine offers unique advantages for biomarker discovery: it is easily obtainable, lacks homeostatic mechanisms, and can reflect early pathological changes. Previous research has demonstrated that urine can mirror brain pathophysiology, with altered protein activities crossing the blood-brain barrier appearing in urine, as shown in studies of occlusive disease, depression, and Parkinson' s disease. Building on prior work measuring escitalopram treatment response, evidence indicates that urinary proteomics can detect early differences in treatment efficacy from the second week, providing potential early biomarkers to predict effective interventions for major depressive disorder.

This study extends previous research on escitalopram efficacy by exploring whether biological pathways enriched in urinary proteins from patients with different treatment responses can reveal potential antidepressant targets and predict therapeutic outcomes. We analyzed pre-treatment urinary proteomes using LC-MS high-throughput mass spectrometry, employing a one-to-many comparison approach (one patient versus a group of healthy controls) to capture individual biological pathways. This strategy addresses clinical heterogeneity by highlighting each patient's unique profile, aligning with precision medicine goals. Our findings suggest that pre-treatment urinary proteomics can guide personalized antidepressant prescribing and identify potential therapeutic targets within altered protein pathways.

Methods

2.1 Participants

This study was approved by the Ethics Committee of Anding Hospital, Beijing (#2017-24), with informed consent obtained from all participants. Samples were collected from Anding Hospital between June and December 2018. The cohort included 20 hospitalized patients with depression, comprising 10 with good escitalopram response and 10 with poor response, plus 9 healthy controls. Detailed participant characteristics and diagnostic information are provided in Huan et al.

2.2 Experimental Design

Raw data were derived from our previous study. We selected 20 patients with major depressive disorder (10 good responders, 10 poor responders) and 9 healthy controls, analyzing quantitative urinary proteome results from pre-treatment samples. Using a one-to-many comparison design, each depressed

patient' s urinary proteome was compared against the healthy control group to identify differentially expressed proteins for biological pathway enrichment. This approach yielded individual patient pathways, which were then pooled by response group (good versus poor) to identify shared and unique pathways. Urine sample processing and high-throughput LC-MS mass spectrometry followed established protocols.

2.3 Data Processing

Mass spectrometry raw data were quantified using Spectronaut, with raw file segments identified via swiss_rat_iRT. Software parameters followed Huan et al. For differential protein selection, fold change (FC) thresholds were 1.5 or 0.67 with p-values <0.05 when comparing individual patients to the averaged healthy control group.

2.4 Functional Analysis

Ingenuity Pathway Analysis (IPA) was used to analyze enriched biological pathways from differential proteins identified between depressed patients and healthy controls. Protein datasets were uploaded to the IPA platform for literature comparison. Biological functions were assigned to each canonical pathway based on functional importance, with pathways selected at $p<0.05$ significance. Venn diagrams were generated using Draw Venn Diagram (ugent.be) to visualize pathway overlaps between response groups.

Results

3.1 Comparison of Urinary Proteomes Between Depressed Patients and Healthy Controls

Participants were recruited from the National Clinical Research Center for Mental Disorders at Beijing Anding Hospital. We collected pre-treatment urine samples from 20 escitalopram-treated depressed patients (10 good responders, 10 poor responders) and 9 healthy controls. Given substantial clinical heterogeneity among patients, group comparisons might mask individual uniqueness and obscure treatment effects. Therefore, we employed a one-to-many comparison strategy, contrasting each depressed patient' s proteome against the healthy control group to capture individual-specific alterations while meeting precision medicine needs. High-throughput LC-MS with data-independent acquisition (DIA) was used for urinary proteome analysis. The number of proteins identified per patient is detailed in Supplementary Tables 1 and 2, with differential protein selection criteria of fold change 1.5 or 0.67 and $p<0.05$.

3.2 Urinary Proteomics Predicts Escitalopram Efficacy

To accurately identify disease-relevant pathways from differential proteins, we performed comprehensive IPA analysis on each patient' s one-to-many compar-

ison results. Biological pathways enriched in good-response and poor-response groups were compiled separately (Supplementary Table 1). The two groups shared 184 pathways, while good responders had 54 unique pathways and poor responders had 94 unique pathways (Figure 2). Unique pathways for each group are listed in Supplementary Tables 2 and 3.

Notably, among pathways unique to good responders, several were enriched in multiple patients: Superpathway of Serine and Glycine Biosynthesis I, Role of IL-17A in Psoriasis, -glutamyl Cycle, Pentose Phosphate Pathway (Non-oxidative Branch), Glycogen Degradation III, HOTAIR Regulatory Pathway, VDR/RXR Activation, and MSP-RON Signaling Pathway (Table 1). In the poor-response group, frequently enriched unique pathways included Role of PKR in Interferon Induction and Antiviral Response, PCP Pathway, HIF1 Signaling, STAT3 Pathway, Tyrosine Biosynthesis IV, 4-aminobutyrate Degradation I, Melatonin Degradation III, and Actin Nucleation by ARP-WASP Complex (Table 2).

Most significantly, the Superpathway of Serine and Glycine Biosynthesis I was enriched in 6 of 10 good responders (60%). Previous studies identified GLDC as a biomarker for citalopram/escitalopram response, and our finding of 60% enrichment exclusively in the good-response group strongly supports urinary proteomics' predictive potential. This consistency is unlikely to be random and demonstrates that urinary proteins can predict escitalopram efficacy.

3.3 Urinary Proteomics Identifies Potential Antidepressant Targets

Pathway enrichment analysis revealed numerous processes related to depression pathophysiology, neurophysiology, neurotransmitter regulation, mood modulation, and cognition (Supplementary Table 1 and 2). We identified several potential antidepressant targets (Table 3). HIF1 signaling may play an important role in antidepressant mechanisms. STAT3 participates in serotonin transporter function and depression-like behavior, with human STAT3 polymorphisms linked to antidepressant response. Thyroid hormones accelerate antidepressant effects and are used clinically. Serotonin receptor signaling is a well-established antidepressant target. Glycine is involved in depression pathophysiology and represents a promising rapid antidepressant target. Ceramide metabolism negatively regulates major depression pathogenesis, with elevated hippocampal ceramide promoting neuroinflammation and degeneration. Antidepressants may exert effects by reducing ceramide through acid sphingomyelinase (aSMase) inhibition, a mechanism exploited by FIASMAs (Functional Inhibitors of Acid Sphingomyelinase).

Discussion

Urinary proteomics has been used to identify biomarkers for depression treatment efficacy and diagnosis. Given the high non-response rate and heterogeneity among depressed patients, effective prediction of individual treatment outcomes

is crucial. Our one-to-many comparison approach captures individual patient specificity, aligning with precision and personalized medicine principles. By analyzing pre-treatment urinary proteomes from patients with divergent escitalopram responses, we demonstrated the potential to predict treatment efficacy.

The unique biological pathways identified in each response group encompass many depression-relevant mechanisms. Poor-response pathways included: (i) dopamine receptor signaling, implicated in anhedonia and motivational deficits; (ii) HIF1 signaling, associated with mood disorders and hypoxia; (iii) STAT3 pathway, linked to serotonin transporter function; (iv) thyroid hormone synthesis, affecting mood and cognition; (v) IL-10 signaling and tyrosine biosynthesis, related to inflammation and neurotransmitter precursors; (vi) chemokine signaling, associated with inflammatory depression phenotypes; and (vii) glutamate pathways, representing emerging antidepressant targets.

Good-response pathways featured: (i) glycine synthesis, essential for NMDAR function and synaptic plasticity, with glycine serving as a clinical marker and rapid antidepressant target; (ii) ceramide degradation, where antidepressants may act by reducing pro-inflammatory ceramide levels; and (iii) glutamate receptor pathways, with NMDAR antagonists like ketamine showing rapid antidepressant effects.

Our findings demonstrate that pre-treatment urinary proteomics can predict escitalopram efficacy and identify therapeutic targets. The glycine pathway enrichment in 60% of good responders replicates previous genetic findings, providing strong validation. While clinical application faces challenges—including small sample size requiring validation and potential ethnic differences in SNP applicability—our Chinese cohort results establish a foundation for predictive urinary proteomics in depression treatment.

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