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Urinary metabolic phenotyping of mucopolysaccharidosis type I combining untargeted and targeted strategies with data modeling

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ABSTRACT

Background: Application of metabolic phenotyping could expand the pathophysiological knowledge of mucopolysaccharidoses (MPS) and may reveal the comprehensive metabolic impairments in MPS. However, few studies applied this approach to MPS.

Methods: We applied targeted and untargeted metabolic profiling in urine samples obtained from a French cohort comprising 19 MPS I and 15 MPS I treated patients along with 66 controls. For that purpose, we used ultra-high-performance liquid chromatography combined with ion mobility and high-resolution mass spectrometry following a protocol designed for large-scale metabolomics studies regarding robustness and reproducibility. Furthermore, 24 amino acids have been quantified using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Keratan sulfate, Heparan sulfate and Dermatan sulfate concentrations have also been measured using an LC-MS/MS method. Univariate and multivariate data analyses have been used to select discriminant metabolites. The mummichog algorithm has been used for pathway analysis.

Results: The studied groups yielded distinct biochemical phenotypes using multivariate data analysis. Univariate statistics also revealed metabolites that differentiated the groups. Specifically, metabolites related to the amino acid metabolism. Pathway analysis revealed that several major amino acid pathways were dysregulated in MPS. Comparison of targeted and untargeted metabolomics data with in silico results yielded arginine, proline and glutathione metabolisms being the most affected.

Conclusion: This study is one of the first metabolic phenotyping studies of MPS I. The findings might help to generate new hypotheses about MPS pathophysiology and to develop further targeted studies of a smaller number of potentially key metabolites.

Abbreviations: IEM, inborn errors of metabolism; LSD, lysosomal storage diseases; MPS, mucopolysaccharidoses; GAGs, glycosaminoglycans; MPS I, mucopolysaccharidosis type I; MPS IT, treated mucopolysaccharidosis type I; UPLC-IM-MS, ultraperformance liquid chromatography-ion mobility mass spectrometry; CCS, collision cross section; ERT, enzyme replacement therapy; QC, quality control; HS, heparan sulfate; DS, dermatan sulfate; KS, keratan sulfate; ROC, receiver operating characteristic; FDR, false discovery rate; PCA, principal component analysis; OPLS-DA, orthogonal partial least-squares-discriminant analysis; VIP, variable influence in projection; AUC, area under curve; mTORC1, mammalian target of rapamycin complex 1

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1. Introduction

Inborn errors of metabolism (IEM) represent a group of about 500 rare diseases with an overall estimated incidence of 1/2500. The diversity of involved metabolisms explains the difficulties in establishing their diagnosis. Optimal management of these patients requires then improved speed of biochemical investigations to allow early diagnosis and better monitoring. The rise of "omic" approaches offered a growing hope to provide new effective tools for screening, diagnosis and monitoring of these diseases. Unlike the conventional medical biology practice based on the sequential study of genes, proteins and metabolites, the great challenge of modern biology is to understand disease as a complex, integrated and dynamic network [1]. The concept of "metabolome" refers to the comprehensive complement of all metabolites present in a given biological system, fluid, cell or tissue [2]. So, metabolomics is one of the "omic" technologies based on biochemical characterizations of the metabolome and its changes related to genetic and environmental factors. Metabolomics allows evaluating the biochemical mechanisms involved cell or tissue changes in a systematic fashion [3,4]. Given the strong link between IEM and metabolism, metabolomics is very appealing to explore these diseases [5]. For years, mass spectrometry has been used to assess IEM [6–9]. However, few metabolomic research has been published in lysosomal storage diseases (LSD) field. LSDs represent a group of about 50 inherited disorders due to lysosomal proteins deficiencies which lead to a progressive accumulation of compounds within the lysosome. This metabolite storage causes various organ failures and premature death [10]. Mucopolysaccharidoses (MPS) belong to the LSD group. They are caused by impaired catabolism of glycosaminoglycans (GAGs), leading to their accumulation in lysosomes and extracellular matrix [11]. Accumulated GAGs cause progressively multiple tissues and organ damages [12]. There are 11 known enzyme deficiencies, resulting in seven distinct forms of MPS [10]. The overall incidence is > 1 in 30,000 live births [13]. Most MPS patients are asymptomatic after birth, however, prenatal symptoms may be observed in MPS I, MPS IVA and more frequently in MPS VII. MPS symptoms and severity vary with patients and MPS subtypes. Several MPS treatments are in clinical use or being investigated under clinical trials for patients [14]. MPS I is a rare autosomal recessive disorder caused by α-L-Iduronidase (IDUA, EC 3.2.1.76) deficiency. IDUA degrades complex polysaccharides by removing a single α-L-iduronyl residue from heparan sulfate and dermatan sulfate. The symptoms range from the severe Hurler form [MPS IH - OMIM #67014] to the more attenuated Hurler–Scheie (MPS IH/S - OMIM #607015) and Scheie (MPS IS - OMIM #67016) phenotypes. The classification is mainly based on the age at first symptoms and the presence or not of mental retardation [15]. The average survival age is of 28 years which imply patient's shift from pediatrics to adults [16]. Two specific treatments are available: hematopoietic stem cell transplantations (from bone marrow or blood cord donors) since the 1980s, and enzyme replacement therapy (ERT) (Laronidase, ALDURAZYME) since the 2000s. The aim of this study is to apply both targeted and untargeted metabolic profiling on MPS I patients compared to controls and to treated MPS I patients (MPS IT) to assess metabolic changes in this condition.

2. Materials and methods

2.1. Urine samples

Random urine samples were collected from MPS patients in whom the diagnosis had been confirmed by demonstrating marked enzyme deficiency in leucocytes and/or by molecular analysis. Pseudodeficiencies have been ruled out. Urine samples were collected within seven reference centers for inherited metabolic diseases in France. Nineteen untreated MPS I patients were evaluated: 18 males (age range from 1 to 43.6 years, mean age: 22 years) and 1 female (age 5.5 years). Control urine samples from 66 healthy subjects, 27 males and 39 females (age range from 5.5 to 70 years, mean age: 40.8 years). Fifteen samples from MPS IT with enzyme replacement therapy, 11 males and 4 females (ages range from 1.3 to 39.3 years, mean age: 11.5 years) were analyzed. This project was approved by the Research Ethics Board of Rouen University Hospital (CERNI E2016-21).

2.2. Metabolic phenotyping

2.2.1. Sample preparation

For untargeted metabolomics, urine samples were processed by transferring 200 μL of urines to 1.5 mL tubes and centrifuged at 4 °C for 10 min at 13,000*g* then 100 μL ultrapure water were added to 100 μL of supernatant and mixed. For amino acids and GAG analysis, detailed protocols are presented in Supporting information.

2.2.2. Untargeted analysis

Ultra-high-performance liquid chromatography-ion mobility mass spectrometry and data-independent MS acquisition with simultaneous analysis of molecular fragmentation (MS^E) were performed on Synapt G2 HDMS (Waters, Saint-Quentin-en-Yvelines, France) mass spectrometer as previously described [17]. Detailed protocol is presented in Supplementary material.

2.2.3. Raw data preprocessing

All LC-IM/MS raw data files, data processing, peak detection and peak matching across samples using retention time (t_R) correction and chromatographic alignment along with drift time and cross collision section (CCS) calculation were performed using Progenesis QI (Waters MS Technologies, Manchester, UK) to yield a data matrix containing retention times, accurate masses (*m/z*), CCS and peak intensities. The preprocessing step resulted in an X-matrix where t_R, CCS and *m/z* values were concatenated into "t_R_m/z_CCS" features (in columns) present in each sample (in rows) with corresponding peak areas.

2.2.4. Quality control

Ten microliters of each urine sample are mixed together to generate a pooled quality control sample (QCs). QCs and mobile phase blank samples were injected sequentially in-between the urine samples. In addition, a dilution series of QC samples (6%, 12.5%, 25%, 50% and 100% of original concentration) are used to assess the quality of the extracted features. More details are presented as Supporting information.

2.2.5. Targeted analysis

2.2.5.1. Amino acids quantification. The analysis of free amino acid profiles in urine was based on a liquid chromatography coupled to tandem mass spectrometry method and the aTRAQ reagent. The aTRAQ kit (Sciex, France) allows to quantify 24 proteinogenic and non-proteinogenic free amino acids, in a range of biological fluids. The detailed description of the applied LC-MS/MS methodology is presented in Supplementary material. The amino acids concentrations were normalized using creatininuria.

2.2.5.2. Glycosaminoglycan quantification (HS, DS and KS). Total urinary GAGs were measured with the dimethyl methylene blue-binding assay [18]. Urinary GAG-derived disaccharides (heparan sulfate, dermatan sulfate and keratan sulfate) were analyzed using LC-MS/MS as described by Langereis et al. [19]. The detailed description of the protocol is presented in Supplementary material. The GAG concentrations were normalized on creatininuria.

2.3. Statistical analyses

A one-way analysis of variance (ANOVA) test was applied to each selected variable in order to confirm their actual difference between the