LC-QTOF-MS BASED TARGETED AND UNTARGETED METABOLOMIC APPROACHES FOR THE IDENTIFICATION OF POTENTIAL BIOMARKERS IN PLASMA FROM PEDIATRICS WITH CHRONIC KIDNEY DISEASE

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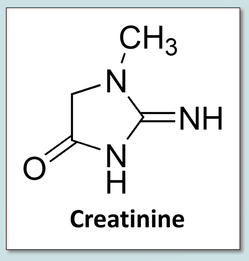
INTRODUCTION

Chronic kidney disease (CKD) is a major worldwide public health problem which causes several disturbances due to an irreversible kidney damage which can progress to renal hypofunction. However, information available for CKD in both pediatric and adult population is limited. As a result, CKD is difficult to diagnose, to follow in progression and to evaluate the response to therapy.



Creatinine

- **"Classic biomarker" in clinical practice**
- **Lacks sensitivity and reveals kidney damage when an important nephronic loss has already taken place**

NEW BIOMARKERS
TO BE USED IN PEDIATRICS ARE NEEDED
- DNA
- RNA
- Proteins
- **Metabolites**

OMICS
Useful sciences for finding new potential biomarkers:
- Genomics
- Transcriptomics
- Proteomics
- **Metabolomics**

METABOLOMICS

Corresponds to the study of small molecules, typically below 1500 Da, in a biological system.

Metabolite levels are considered the ultimate response of biological systems to genetic or environmental changes.

DIFFERENT APPROACHES

TARGETED METABOLOMICS

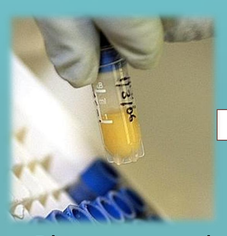
- Determination and quantification of known metabolites, suspicious to be altered in a disease
- More complex and specific sample treatment and analytical method

UNTARGETED METABOLOMICS

- Measurement and comparison of as many metabolites as possible without bias in control and disease groups to find unknown metabolites to be used as new potential biomarkers
- General and more simple sample treatment and analytical method

EXPERIMENTAL

TARGETED METABOLOMICS
-32 CKD samples
-24 control samples
* Calibration samples + QC samples

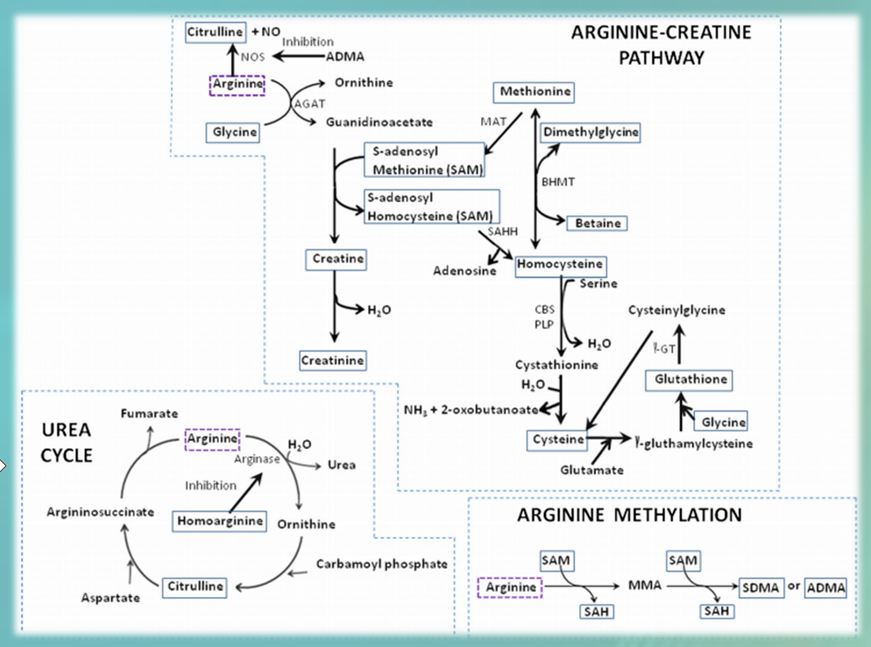


Aliquoted and divided for different sample treatments

Plasma samples:
32 from CKD pediatrics (3-18 y)
29 from control pediatrics (6-19 y)

Information available: age, sex, CKD stage, treatment (not treated, dialyzed, transplanted)

UNTARGETED METABOLOMICS
-32 CKD samples
-26 control samples
* QC samples



- **Development and validation** of an ion-pairing LC-QTOF-MS methodology
- Quantification of **16 metabolites** from **the arginine-creatine metabolic pathway, arginine methylation and urea cycle** in plasma using analytical standards
- **Univariate and multivariate analysis** of the results to find significantly altered metabolites in CKD in comparison with control patients
- **Published work**:
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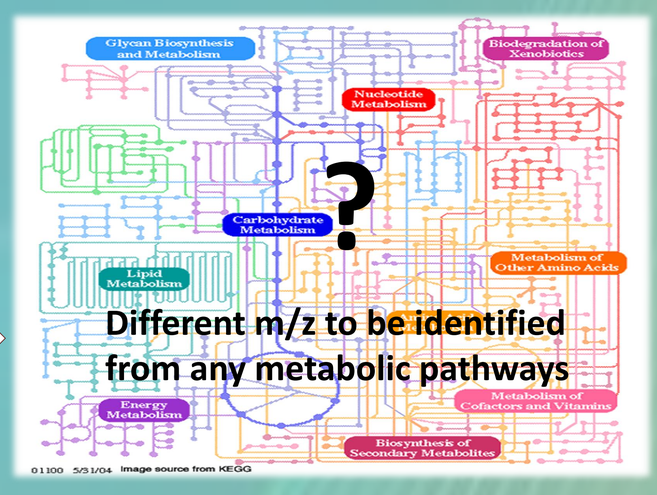

- **Development** of a reverse-phase LC-QTOF-MS methodology
- Measured **as many entities as possible**
- There is **no need for analytical standards** (except for identification/confirmation)
- **Workflow**: data pre-processing (noise filtering, peak detection, peak alignment), data analysis (chemometric and statistical analysis to find unknown metabolites whose levels are statistically different in control and CKD pediatrics) and identification of entities using different databases, MS/MS fragmentation analysis and identity confirmation by means of analytical standards.

RESULTS AND CONCLUSIONS

- **Targeted metabolomics approach**: Univariate analysis showed that glycine, citrulline, creatinine, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) were significantly increased for CKD pediatric patients. Similarly, regarding multivariate analysis, S-adenosylhomocysteine, SDMA, creatinine, citrulline, S-adenosylmethionine (SAM), ADMA, glutathione, dimethylglycine and glycine were found to be increased in pediatrics with CKD. Moreover, PCA showed that both groups are well separated and it is possible to predict the early stages of the disease with a more than 10 % better accuracy in comparison with the use of creatinine only including these analytes.
- **Untargeted metabolomics approach**: Around 15 entities were found to be significant after doing data pre-processing and subsequent data analysis. Identification of these entities using different databases and MS/MS fragmentation analysis is being performed.

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