様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Predicting asthma exacerbations through longitudinal metabolomics

研究課題名（英文）Predicting asthma exacerbations through longitudinal metabolomics

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：喘息は、主要な非感染性疾患の1つであり、世界中で2億人以上の患者がいます。 病気の悪化は、患者にとって大きな負担ですと、健康とリスク予測を監視する包括的アプローチも欠けています。 このプロジェクトでは、12人の喘息患者と12人の健康対照者から、数か月間収集した800を超える尿サンプル中の化合物を測定しました。 データ分析は、まだ進行中ですが、個々のデータでは、喘息の悪化に関連する尿代謝プロファイルといくつかの化合物の変化を示しています。個々の生物学的変動を理解することは、病気の悪化を防ぐための健康モニタリングに活用できます。

研究成果の学術的意義や社会的意義
This project enabled creation of the tools needed for cohort scale urine metabolomics measurements with high precision. Reliable data is necessary to find chemical changes related to disease exacerbations. From several hundreds of detected urinary compounds few show shifts related to health status.

研究成果の概要（英文）：Asthma is one of the major noncommunicable diseases, affecting over 200 million people globally. Disease exacerbations are a major burden for asthmatics, however comprehensive approaches to monitor health and risk prediction are lacking. In this project we have measured chemical compounds in over 800 urine samples collected longitudinally over several months from 12 asthmatics and 12 healthy controls. Data analysis is still ongoing, but individuals show distinct urinary metabolic profiles and several chemical compounds show shifts related to asthma exacerbations. Understanding biological fluctuations in individuals can be used for health monitoring to prevent exacerbation.

研究分野：呼吸器内科学関連

キーワード：喘息　尿　メタボロミクス　asthma　urine　metabolomics

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Globally <235 million people have asthma with ~3 million asthmatics in Japan according to the WHO. In asthma, acute flare-ups of severe symptoms and rapidly increasing obstruction called 'asthma exacerbations' occur episodically, which are mostly due to respiratory viral infections, especially by rhinovirus. This potentially causes emergency visits and hospitalization requiring urgent medical treatment. Attempts to use clinical markers to predict the severity of exacerbations, based on single time point assessment using univariate and multivariate clinical models have only been partially successful. Prediction models of (the severity of) asthma exacerbations based on clinical markers tend to over-estimate the average risk of a population, thereby insufficiently predicting the risk of a given individual. Longitudinal measurements are needed for identification of a patient's individual severity of exacerbation. Such periodic monitoring includes: the history of exacerbations and time series of signs, symptoms and repeated monitoring of lung function measures (as peak flow, spirometry etc.). Although recommended for the diagnosis and management of asthma (www.ginasthma.org), these clinical measures alone cannot adequately cover the heterogeneity of asthma sub-phenotypes. The latter requires capturing the complex underlying biology, which can best be addressed by a system medicine approach combining clinical and omics data. In this project we perform cutting-edge molecular phenotyping of urine from a unique and well characterized asthma longitudinal cohort. Temporal assessment of novel biomarkers in non-invasive biofluids such as urine is a crucial step forward to monitor and predict the risk/severity of asthma exacerbations.

２．研究の目的

- To compare temporal fluctuations in urinary metabolites between asthmatics and healthy controls, and establish reference ranges for urinary metabolites.
- To investigate the change in variations of metabolite profiles following experimental rhinovirus infection.
- To model and predict the severity of rhinovirus-induced asthma exacerbations based upon time series analysis of biomarkers.

３．研究の方法

Longitudinal asthma cohort. Urine samples were collected from asthmatics (n=12) and controls (n=12) over the time course of three months (total 842 samples) including a rhinovirus challenge (Fig. 1).

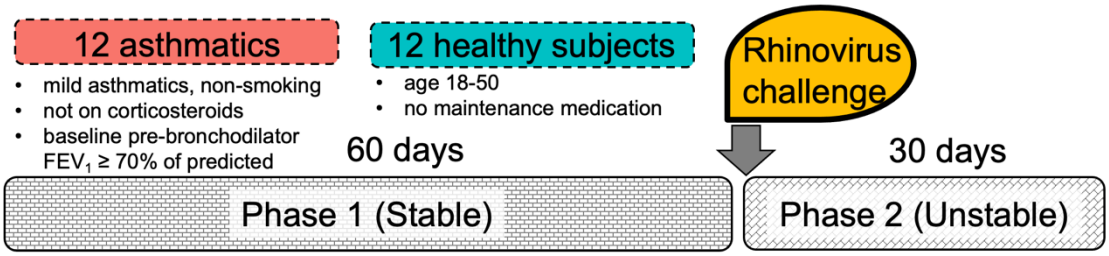

Figure. 1. Longitudinal asthma cohort study overview.

Untargeted metabolic profiling of urine samples. Samples were aliquoted into 96-well plates and processed using a Bravo automated liquid-handling platform (Agilent Technologies, Inc.) equipped with a cooling unit set at 4 °C. Specific gravity (SG) measurements based on refraction were performed and each urine sample was diluted with water to a common SG value of 1.002. SG normalized samples were extracted with acetonitrile containing technical internal standards (tIS). Untargeted metabolomics data were acquired using three liquid chromatography – mass spectrometry (LC-MS) methods. Briefly, samples were measured on an Agilent 1290 Infinity II ultrahigh-performance liquid chromatography system coupled to a 6550 iFunnel quadrupole-time-of-flight mass spectrometer equipped with a dual AJS electrospray ionization source tuned for the 50−750 *m/z* range. HILIC chromatography at acidic pH (pH =2.6) was adapted from Chaleckis et al. [1] using a SeQuant ZIC-HILIC column

and a gradient between (A) water containing 0.1% formic acid (pH = 2.6) and (B) acetonitrile containing 0.1% formic acid. The separation gradient included an isocratic step at 95% B for 1.5 min followed by a gradient to 40% B in 10.5 min. HILIC chromatography at basic pH (pH=9.3) was run on a SeQuant ZIC-pHILIC column with (A) ammonium acetate 5 mM with 0.04% ammonium hydroxide in water (pH = 9.3) and (B) pure acetonitrile as mobile phases. The gradient was set at 88–60% B from 0 to 8.5 min and the column oven was heated at 35 °C. The acquisition was performed in DIA mode using a mass range of 40–1200 *m/z* with three different collision energies (0, 10, and 30 eV) in positive ionization mode for the HILIC chromatography at acidic pH and in both, positive and negative, ionization modes for the HILIC chromatography at basic pH.

Untargeted metabolomics data and identification of urinary metabolites. LC-MS raw data files were converted to mzML format using ProteoWizard and an initial quality check was performed in MZmine 2. For processing of the raw data to annotated datasets MS-DIAL 4 software was used. In MS-DIAL the MS2 spectra were deconvoluted using MS2Dec and CorrDec algorithms. Identifications were based on in-house compound libraries containing >400 chemical standards. Metabolite annotations by MS-DIAL were assessed using the following criteria: a RT shift <0.5 min, a mass shift <10 mDa, and, for spectral match, a dot product score without weighting above 800 and at least two matching MS2 peaks with the reference spectra to avoid spurious high scores from too low number of peaks. Any annotations that did not fulfill one of the RT or m/z criteria, but were still determined to be accurate receive an explanatory comment in the identification tables. Peak areas were exported from MS-DIAL., Coefficients of variation (CVs), mean peak intensities and other parameters to accesses data quality were computed using R scripts. Datasets containing identified metabolites as well as all detected features were used for exploratory analyses. Before analyses dataset signals were corrected for measurement drift using a Matlab algorithm based upon the SQC (study quality control) sample signals. Data analyses of metabolite shifts and responses to rhinovirus challenge are underway using R scripts.

4．研究成果

Data quality of the untargeted metabolomics datasets. To obtain high quality untargeted metabolomics data we have developed an automated urinary metabolomics workflow [2] (Fig.2). Dilution of the urine samples to the same SG and automation enabled high precision measurements.

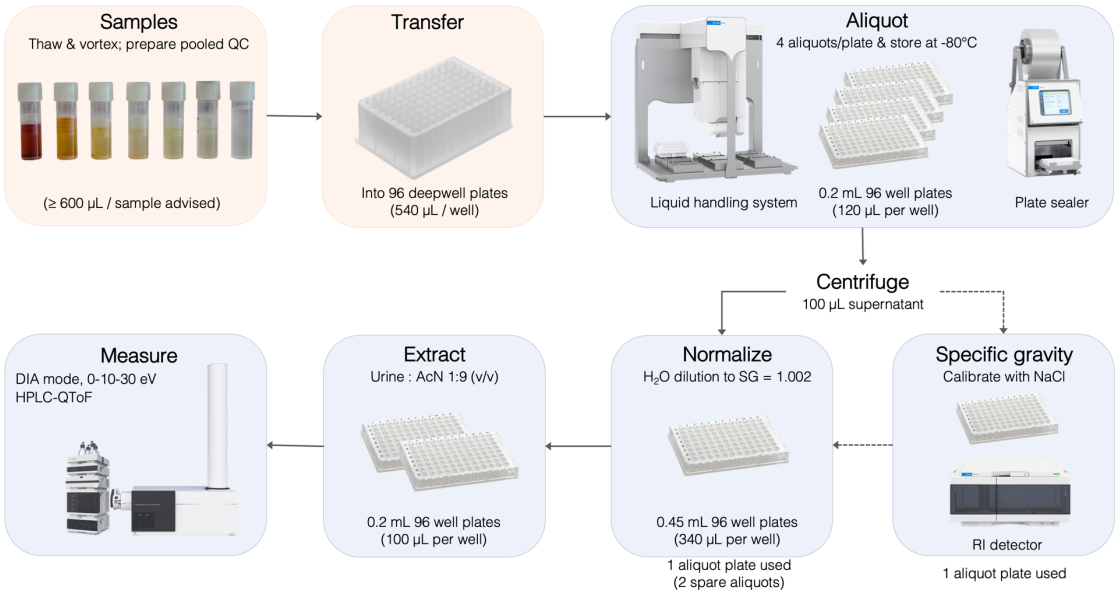

Figure. 2. Urinary metabolomics workflow.

CVs of the 4 from 5 tIS in the analytical batches were <15% and <20% in the SQCs and study samples respectively (Table 1) showing high precision of the methods. Two internal standards, fluorocytosine and tricarballylic acid, did not perform very well at large-scale because of abundant compounds eluting nearby or ion suppression/enhancement.

Table 1. CVs of the tIS in the datasets.

ZIC-HILIC column positive ionization	Mean CV_{QC} (min-max)	Mean CV_{sample} (min-max)
Pyrantel	2.7 (1.0 - 4.8)	3.7 (1.5 - 6.8)
CHES	3.6 (1.4 - 6.9)	6.8 (4.1 -16.0)
Fluorocytosine	8.5 (2.3 - 19.0)	16.0 (5.5 - 31.0)
PIPES	4.4 (1.7 - 9.2)	6.7 (3.1 - 13.0)
HEPES	4.5 (1.5 - 9.2)	6.2 (1.9 -15.0)
ZIC-pHILIC column negative ionization	**Mean CV_{QC} (min-max)**	**Mean CV_{sample} (min-max)**
Fluorocinnamic acid	5.9 (2.8 - 12.0)	12.5 (5.9 - 18.0)
CHES	4.4 (2.7 - 7.0)	6.9 (4.9 - 11.0)
HEPES	5.3 (1.5 - 7.9)	9.4 (6.7 - 12.0)
PIPES	4.7 (2.4 - 7.4)	10.0 (6.2 - 16.0)
Tricarballylic acid	12.4 (6.8 - 18.0)	36.5 (14.0 - 66.0)

Metabolite coverage and annotation. To provide a broader coverage of polar urine components we chose two complimentary chromatographic methods as well as positive and negative ionization modes. CorrDec algorithm [3] was developed to obtain MS2 spectra in the multisample datasets. To obtain reliable identifications we have used in-house constructed chemical libraries [4]. This allowed us to identify >200 metabolites across the urinary datasets based on chemical libraries and have putative identifications for further >300 metabolites for which we currently lack chemical standards.

Temporal fluctuations of urinary metabolites. Preliminary results show that in >50% of the study participants cytosine levels spiked after rhinovirus challenge (Fig. 3). A modified robust regression analysis technique, LASSO (least absolute shrinkage and selection operator) showed decreases of carnitine species related to the rhinovirus challenge. Analysis of the datasets is still ongoing.

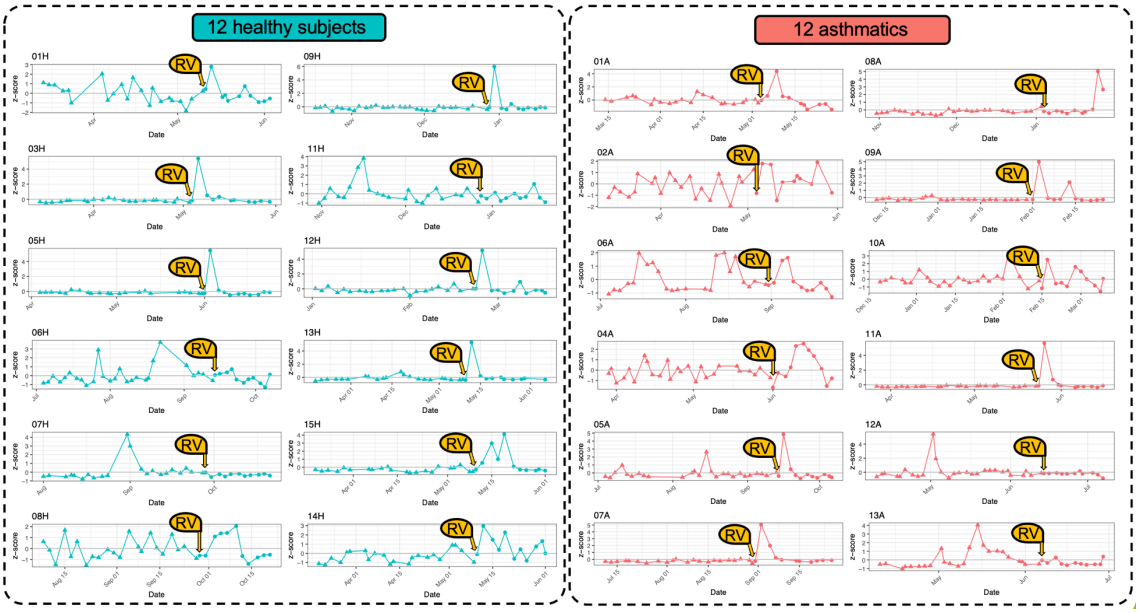

Figure 3. Urinary cytosine levels in the study participants throughout the duration of the study (RV – rhinovirus challenge).

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[1] **Chaleckis, R.**; Naz, S.; Meister, I.; Wheelock, C.E. LC-MS-Based metabolomics of biofluids using all-ion fragmentation (AIF) acquisition. *Methods Mol. Biol.* 1730, 45–58.

[2] Meister, I.; Zhang, P.; Sinha, A.; Skold, C.M.; Wheelock, A.M.; Izumi, T.; **Chaleckis, R.***; Wheelock, C.E. * High-Precision Automated Workflow for Urinary Untargeted Metabolomic Epidemiology. *Anal. Chem.* **2021**, *93*, 5248-5258.
* equal contribution

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* co-first

[4] Tada, I.; Tsugawa, H.; Meister, I.; Zhang, P.; Shu, R.; Katsumi, R.; Wheelock, C.E.; Arita, M.; **Chaleckis, R**. Creating a Reliable Mass Spectral-Retention Time Library for All Ion Fragmentation-Based Metabolomics. *Metabolites* **2019**, *9*.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　4件）

１．著者名 Tada Ipputa、Tsugawa Hiroshi、Meister Isabel、Zhang Pei、Shu Rie、Katsumi Riho、Wheelock Craig E.、Arita Masanori、Chaleckis Romanas	４．巻 9
２．論文標題 Creating a Reliable Mass Spectral Retention Time Library for All Ion Fragmentation-Based Metabolomics	５．発行年 2019年
３．雑誌名 Metabolites	６．最初と最後の頁 251～251
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/metabo9110251	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Zhang Pei、Arora Manish、Chaleckis Romanas、Isobe Tomohiko、Jain Mohit、Meister Isabel、Melen Erik、Perzanowski Matthew、Torta Federico、Wenk Markus R.、Wheelock Craig E.	４．巻 9
２．論文標題 Tackling the Complexity of the Exposome: Considerations from the Gunma University Initiative for Advanced Research (GIAR) Exposome Symposium	５．発行年 2019年
３．雑誌名 Metabolites	６．最初と最後の頁 106～106
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/metabo9060106	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Tada Ipputa、Chaleckis Romanas、Tsugawa Hiroshi、Meister Isabel、Zhang Pei、Lazarinis Nikolaos、Dahlen Barbro、Wheelock Craig E.、Arita Masanori	４．巻 92
２．論文標題 Correlation-Based Deconvolution (CorrDec) To Generate High-Quality MS2 Spectra from Data-Independent Acquisition in Multisample Studies	５．発行年 2020年
３．雑誌名 Analytical Chemistry	６．最初と最後の頁 11310～11317
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.analchem.0c01980	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Meister Isabel、Zhang Pei、Sinha Anirban、Skold C. Magnus、Wheelock Asa M.、Izumi Takashi、Chaleckis Romanas、Wheelock Craig E.	４．巻 93
２．論文標題 High-Precision Automated Workflow for Urinary Untargeted Metabolomic Epidemiology	５．発行年 2021年
３．雑誌名 Analytical Chemistry	６．最初と最後の頁 5248～5258
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.analchem.1c00203	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　1件）

1．発表者名 Romanas Chaleckis, Anirban Sinha, Isabel Meister, Pei Zhang, Rene Lutter, Peter J. Sterk, Craig E. Wheelock
2．発表標題 Longitudinal metabolite fluctuations in urine reveal individual variations and responses to rhinovirus challenge (BIOFLUC study)
3．学会等名 Metabolomics 2019（国際学会）
4．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関			
オランダ	University of Amsterdam			
スウェーデン	Karolinska Institutet			