様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Integrative Metabolomics and Exposomics Study of Severe Asthma in Adults

研究課題名（英文）Integrative Metabolomics and Exposomics Study of Severe Asthma in Adults

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研究成果の概要（和文）：このプロジェクトでは、コホートレベルの自動血液サンプル準備プラットフォームが確立され、既存のメタボロミクス/リピドミクスワークフローに組み込まれました。確立されたワークフローを利用して、重症喘息の分子表現型分類が行われた。メタボロミクスおよびリピドミクス、ならびにターゲットスフィンゴ脂質測定は、570の重度の喘息患者および570の対照に対して実施されました。データ分析はまだ進行中ですが、予備結果では、重度の喘息の多くの代謝物が対照群と比較して無秩序であることが示されました。このプロジェクトの結果は、重症喘息の分子メカニズムの説明に役立ち、重症喘息の新薬標的の発見に貢献する可能性があります。

研究成果の学術的意義や社会的意義
Asthma is a chronic inflammatory disease that affects over 300 million people worldwide, which makes it a both financial and medical burden for many countries. Understanding the molecular mechanism of this disease is of critical importance for the discovery of potential treatment targets.

研究成果の概要（英文）：In this project, automated blood sample preparation platforms on cohort level (> 1,000 samples) were established and incorporated into the existing untargeted metabolomics/lipidomics workflow. The methods are very robust with coefficient variance of internal standards in quality control samples below 10%. Utilizing the established workflow, molecular phenotyping of severe asthma was conducted. Untargeted metabolomics and lipidomics as well as targeted sphingolipids measurements were performed on 570 severe asthmatics and 570 controls. Although data analysis is still ongoing, preliminary results showed that many metabolites in severe asthma were disregulated compared to controls, for example, cortisol was down-regulated in asthmatics, sphingosine, taurine and sphingosine-1-phosphate increased in asthmatics. Results from this project could benefit illustration of the molecular mechanism of severe asthma and contribute to new drug target discovery of severe asthma.

研究分野：生体の 構造と 機能お よ び そ の 関連分野

キーワード：Asthma　Metabolomics　Lipidomics

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Asthma is a chronic inflammatory disease characterized by airflow restriction and various respiratory symptoms. Globally, ~300 million people have asthma and this number is rising. There are ~3 million asthmatics in Japan according to the WHO. However, despite this high rate of asthma incidence, there are no molecular descriptors of the different sub-phenotypes of asthma and no clear understanding of the molecular etiology of the disease. There for the key scientific questions are: 1) what are the underlying molecular mechanisms of severe asthma? 2) Can we identify molecular descriptors of asthma sub-phenotypes? To resolve these questions,

２．研究の目的

There are primarily three objectives of this project:
(1) Identify and confirm key metabolic pathway(s) in the pathophysiology of severe asthma.
(2) Define molecular descriptors of asthma sub-phenotypes.
(3) Explore the role of sphingolipids in the pathophysiology of severe asthma.

３．研究の方法

(1) **Cohort design.** Subjects were selected from Partners Biobank. An individual with severe asthma was defined by all of the following: 1) an asthma diagnosis using natural language processing in the Partners electronic medical record; 2) multiple prescriptions for an inhaled steroid medicine, and 3) at least one prescription of oral steroid. Control individuals were identified by the following: 1) no diagnosis of asthma; 2) no inhaled or oral asthma medications; 3) matched on age and BMI. All individuals were Caucasian and did not smoke. Eventually, 570 severe asthmatics and 570 controls were selected. Serum samples were collected from recruited subjects.

(2) **Database construction.** To facilitate the compound annotation in untargeted metabolomics, there is a need to expand our current metabolite database. By referring to existing literature and looking into our own data, a list of 393 metabolites was generated. All the standards of these metabolites were purchased and prepared into stock solutions. Series dilutions of the stock solution were injected to our liquid chromatography – quadrupole time of flight – mass spectrometry (LC-Q/TOF-MS) system for compound characterization. Accurate mass, retention time and fragments of each compound were collected and compiled into a MSP file which could be then used in the MS-DIAL software for metabolite annotation.

(3) **Untargeted metabolic profiling using hydrophilic interaction liquid chromatography.** An aliquot of 20 uL of serum were extracted with 120 uL of methanol containing 6 internal standards. Supernatant obtained after centrifuge was evaporated, and the residues were re-suspended in 80% acetonitrile in water. The supernatant was injected to a zic-HILIC column on the Agilent LC-Q/TOF-MS system. Quality control (QC) acquired by pooling equal amount of each sample was produced and prepared together with actual samples. QCs were injected every 5 samples to monitor the instrument performance. Separation of metabolites was achieved on a zic-HILIC column (100 × 2.1 mm, 3.5 μm particle size; Merck, Darmstadt, Germany) by using an Agilent 1290 HPLC system (Agilent Technologies, Santa Clara, CA, USA). Data were acquired in positive ionization mode on an Agilent 6550 Q-TOF system (Agilent Technologies, Santa Clara, CA, USA) with a mass range of 40−1200 m/z in all ion fragmentation (AIF) mode with three collision energies (0, 10, and 30 eV). Raw data was acquired by MassHunter workstation (Ver. 8.0, Agilent Technologies, Santa Clara, CA, USA).

(4) **Untargeted lipidomic profiling using reverse phase chromatography.** An aliquot of 20 uL of serum were extracted firstly with 60 uL of methanol, then 200 uL of methyl-tert-butyl ether (MTBE) were added to the mixture, and lastly 60 uL of water were added to facilitate the formation of phase separation. After centrifuge, 100 uL of the upper phase were taken for evaporation. The obtained residues were reconstituted with 100 uL of isopropanol. Eventually, the supernatant of this isopropanol dissolved lipid solution were taken for instrument analysis. Separation of lipids was achieved on an Agilent Zorbax Eclipse Plus C18 column (RRHD, 2.1 x 100mm, 1.8 Micron) by using an Agilent 1290 HPLC system (Agilent Technologies, Santa Clara, CA, USA). Mobile phase A was acetonitrile:water (60:40, v/v, 10 mM ammonium formate), and mobile phase B was isopropanol:acetonitrile (90:10, v/v, 10 mM ammonium formate). Data were acquired in both positive and negative ionization modes on an Agilent 6550 Q-TOF system (Agilent Technologies, Santa Clara, CA, USA) with a mass range of 40 −1200 m/z in data dependent acquisition (DDA) mode with the collision energy of 20 V in positive and 25 V in negative mode. Raw data was acquired by MassHunter workstation (Ver. 8.0, Agilent Technologies, Santa Clara, CA, USA).

(5) **Targeted metabolite measurement of sphingolipids.** An aliquot of 25 uL of serum were added with 10 uL of sphingolipid internal standard mixture. After vortex and equilibration, the mixture was extracted with 250 uL of methanol. The extraction was facilitated with vortex and sonication. After centrifuge, the supernatant was transferred and placed to the instrument for analysis. Two QCext were extracted with each batch. A blank sample, consisting of only methanol was also extracted to control for possible contributions of the experimental materials to the observed sphingolipid levels. Before the analyses, 80 QC samples were extracted and the resulting extracts were pooled to be used as QCinj. The pooled samples were aliquoted in injection vials and stored at -80°C to check the reproducibility of the quantitation throughout the analyses. The metabolite separation was achieved on an Agilent Zorbax Eclipse Plus C18 column (RRHD, 2.1 x 100mm, 1.8 Micron) by a Waters Acquity i-Class UPLC Binary Solvent Manager. Mobile phase A was 5% ammonium formate and 0.2% formic acid in water and B was 5% ammonium formate and 0.2% formic acid in methanol. Mass data was acquired on a Waters Xevo TQ-S Triple Quadrupole Mass Spectrometer system using multiple reaction mode (MRM).

(6) **Data processing and analysis.**

① Untargeted metabolomics data. For quick data quality check, raw data files were transformed to mzML format by ProteoWizard (Ver. 3.0) and imported to MZmine (Ver. 2.40). Peak area, peak retention time and peak m/z of internal standards were extracted. The following calculations were conducted: 1) Coefficient variance (CV) of peak area of internal standards in QCs and samples; 2) Retention time stability of internal standards across all samples; and 3) Mass accuracy of internal standards. For data analysis, raw data files were transformed to abf format by using Analysis Base File Converter and loaded to MS-DIAL (Ver. 4.12). Potential retention time shift was corrected in MS-DIAL using internal standards as references. All aligned features were manually checked to minimize problematic peak integration or misalignment. The constructed database mentioned above was used for metabolite annotation.

② Targeted sphingolipid data. Targetlynx files were created for peak integration and quantification. Quantification files were created for each calibration curve batch. Compounds were split in two blocks for processing purposes. Peak integrations were revised for each compound in each sample on a blinded way. Finally the dataset was exported and summarized.

4．研究成果

(1) **Data quality of untargeted HILIC metabolomics.** As it is shown in Figure 1, the CV of peak area internal standards in QCs were mostly below 10% and in samples below 15%, indicating very robust sample extraction and instrument performance. The mass accuracy of internal standards was also calculated and all of them were below 5 ppm.

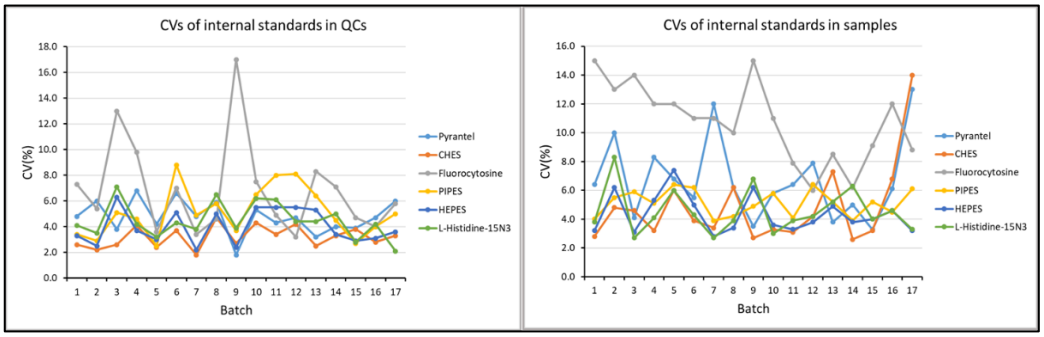

Figure 1. CVs of internal standards in QCs and Samples

(2) **Data quality of untargeted lipidomics.** The internal standards used in lipidomics are structurally very similar to endogenous lipids, which means peak intensities of these compounds are easily affected by the fluctuation of endogenous lipids in different samples. Therefore, we only evaluated the performance of internal standards in QC samples which are in theory identical. Figure 2 shows that all the CV of internal standards were below 10%, indicating a very stable and robust instrument performance.

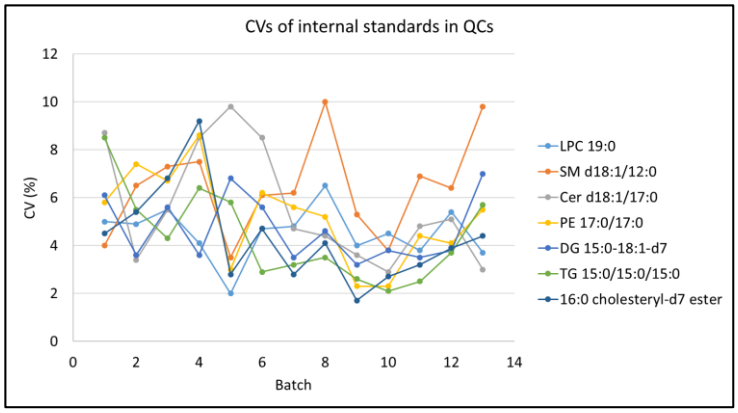

Figure 2. CVs of internal standards in QCs

(3) **Data quality of targeted sphingolipid measurement.** CV of 77 measured sphingolipids were calculated and as Figure 2 shows, 69/77 were below 10% and 76/77 were below 15%. This indicates that the method is robust and reliable.

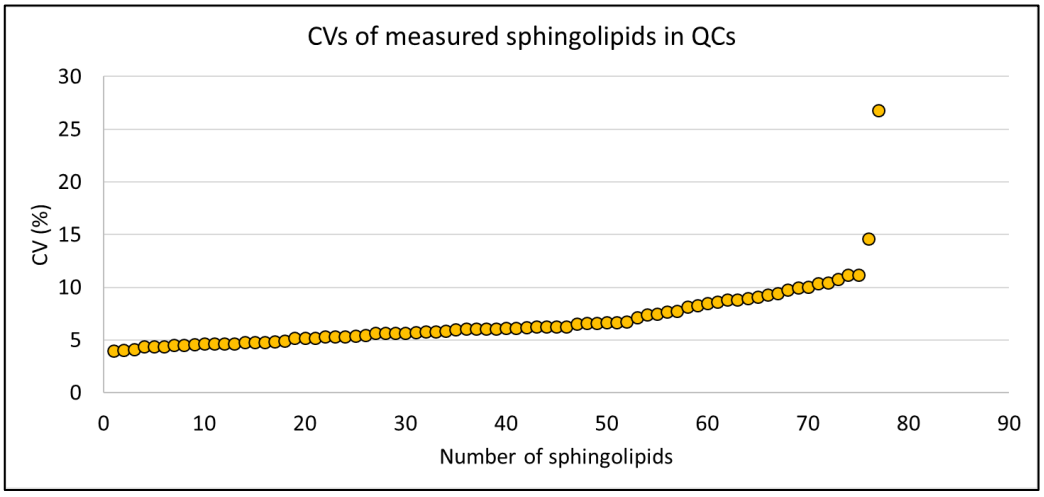

Figure 3. CVs of measured sphingolipids in QCs

(4) **Multiple metabolites were dysregulated in severe asthmatics.** From HILIC data, we found that many metabolites was dysregulated in severe asthmatics including taurine, kynurenine, cortisol and hydroxykynurenine etc. Boxplots of these metabolites are shown in Figure 4. The underlying mechanism of these metabolic shifts should be further investigated in the future.

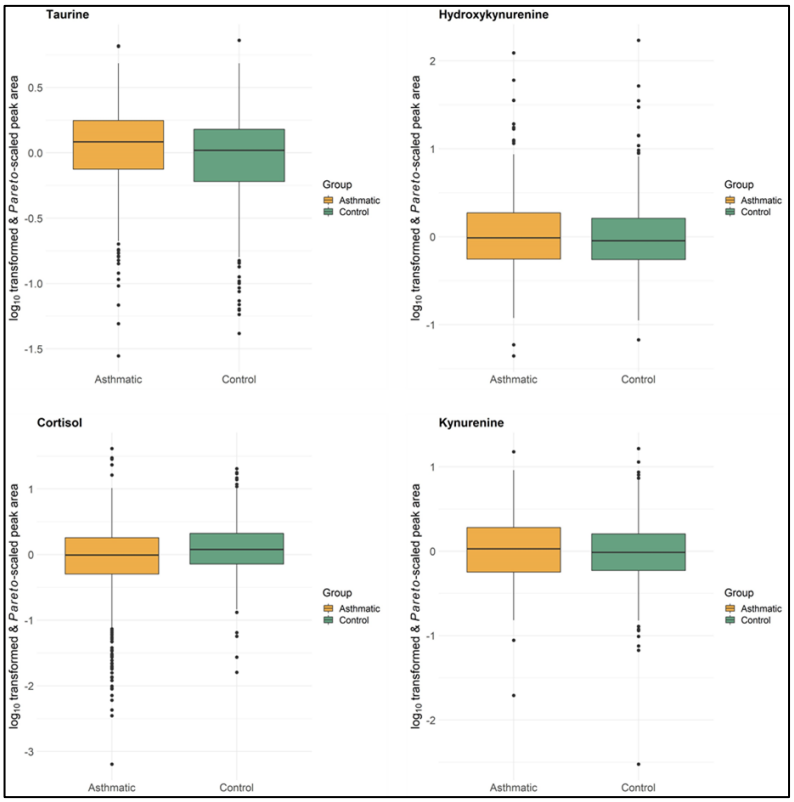

Figure 4. Dysregulated metabolites.

(5) **Multiple sphingolipids were shifted in severe asthmatics.** In the sphingolipid data, we found that sphingosine and sphingosine-1-phosphate etc were upregulated in severe asthmatics.

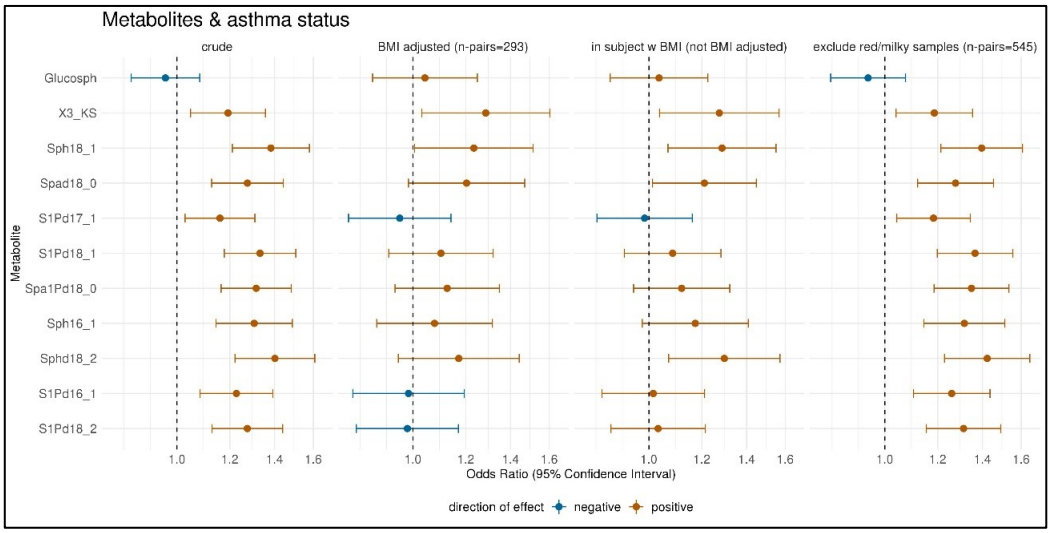

Figure 5. Dysregulated sphingolipids

5．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　2件）

1．著者名 Romanas Chaleckis, Isabel Meister, Pei Zhang and Craig E Wheelock	4．巻 55
2．論文標題 Challenges, progress and promises of metabolite annotation for LC-MS-based metabolomics	5．発行年 2019年
3．雑誌名 Current Opinion in Biotechnology	6．最初と最後の頁 44-50
掲載論文のDOI（デジタルオブジェクト識別子） doi.org/10.1016/j.copbio.2018.07.010	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1．著者名 Zhang Pei、Arora Manish、Chaleckis Romanas、Isobe Tomohiko、Jain Mohit、Meister Isabel、Mel?n 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、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

〔学会発表〕　計0件

〔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
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6．研究組織（つづき）

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研究協力者	チェカー　アントーニーオー (Checa Antonio)	Ｋａｒｏｌｉｎｓｋａ　Ｉｎｓｔｉｔｕｔｅｔ	