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Identification of Lipid Biomarkers in CAD using a Targeted Liquid Chromatography Mass Spectrometry Approach

Akash Kumar Bhaskar [1,2], Swati Varshney [1,2], Khushboo Adlakha[1], Mainak Dutta[3], Akanksha Singh[4], Dipankar Malakar[4], Manoj Pillai[4], Shantanu Sengupta [1,2]

[1]CSIR-Institute of Genomics and Integrative Biology, New Delhi, India. [2]Academy of Scientific & Innovative Research, New Delhi, India. [3]Birla Institute of Technology Pilani, Dubai, United Arab Emirates. [4]SCIEX, 121, Udyog Vihar, Phase IV, Gurgaon, Haryana, India.

ABSTRACT

Coronary artery disease (CAD) has developed as a major public health problem worldwide especially in developing countries like India. The traditional risk factors are useful in identifying people at high risk but lack the desired predictive accuracy. It is therefore important to identify newer biomarkers. CAD is often referred as a metabolic disorder associated with changes in circulating lipids such as triglycerides, cholesterol etc. It is also important to know which particular lipid species has altered, to better understand the disease pathogenesis. Herein we have performed targeted plasma lipidomics approach using liquid chromatography mass spectrometry with scheduled MRM for more than 1000 lipids intended for the sensitive detection of lipid metabolites with robustness, reliability and reproducibility, enabling biomarker research.

INTRODUCTION

Coronary artery disease is one of the major causes of global mortality and morbidity. Conventional markers do not add prognostic value to disease identification therefore it is important to identify newer markers for complex cardiometabolic diseases. CAD is often considered as a metabolic disorder associated with changes in circulating lipids but varying degree of chemical complexity of lipids make their identification challenging. Lipid compositions of biofluids such as plasma are complex, reflecting a wide range of diversity and concentration of different lipid classes.

So, there is a need for robust, rapid and economical high throughput analytical methods that can detect maximum number of lipid species present in plasma and help in better understanding of lipid metabolism in biological systems. LC-MS/MS emerged as a powerful tool for identification and quantification of lipids at species level and can detect very minute amount of metabolite. It has replaced traditional methods for quantitation of lipid like TLC because of its ability to analyze a wider range of compounds in a single analysis.

Here we have used the power of QTRAP® 6500+ System to develop a method where transitions of positive and negative polarity was captured in single run. Instead of using different extraction procedure for isolating phospholipids, neutral lipids, we have used a simple extraction procedure to isolate different types of lipid classes. This new simplified sample preparation in combination with gradient LC, and *Scheduled* MRM™ Algorithm detection allows detecting more than 1000 lipid species.

MATERIALS AND METHODS

Sample Preparation:
Lipid from 10ul of plasma with spiked internal standard was extracted using a mixture dichloromethane/methanol/water (2:2:1 v/v). The extract was finally dissolved in 100% ethanol.

HPLC Conditions:
An ExionLC™ system with a Waters AQUITY UPLC BEH HILIC 1.7um, 2.1x100mm column at 35° C with a gradient of eluent A water/acetoitrile (5/95) + 10mM ammonium acetate, pH-8 and eluent B water/acetonitrile (50/50) + 10mM ammonium acetate, pH-8 was used at a flow rate of 0.5mL/min. The injection volume was set to 10µL and 100% IPA was used for needle wash. Total LC runtime was 16 minutes.

MS/MS Conditions:
A SCIEX QTRAP® 6500+ LC-MS/MS system with improved polarity switching, Turbo V™ source and Electrospray Ionization (ESI) probe was used. Using scheduled MRM, 621 transitions and 646 transitions were scanned in positive mode and negative mode respectively. Every sample was injected in 4 technical replicates analyzing transitions in both positive and negative polarity in a single run. *Scheduled* MRM™ algorithm was used for best accuracy and reproducibility. MultiQuant ™ Software 3.0.2 was used for the quantitative data analysis.

RESULTS

A method for identification and quantitation of 20 lipid classes: sphingomyelin (SM), ceramide (Cer), cholesterol ester (CE), Monoacylglycerol (MAG) diacylglycerol (DAG), Triacylglycerol (TAG), lysophosphatidic acid (PA), phosphotidic acid (PA), lysophosphatidylcholine (LPC), phosphatidylcholine (PC), lysophosphatidylethanolamine (LPE), phosphatidylethanolamine (PE), lysophosphatidylinositol (LPI), phosphatidylinositol (PI), lysophosphatidylglycerol (PG), phosphatidylglycerol (PG), lysophosphatidylserine (LPS), phosphatidylserine (PS), and free fatty acid (FFA) was developed. Reproducibility of method was checked using different plasma samples (Figure 3 and 4).

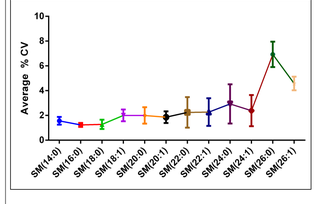

Figure 1. Represent the reproducibility of method for sphingomyelin. Y-axis shows the coefficient of variance for nine different samples each having 4 technical replicate (n=36).

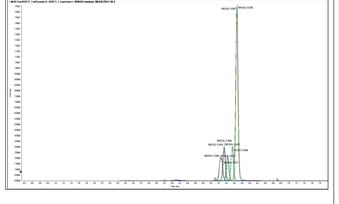

Figure 2. Extracted Ion Chromatogram for Sphingomyelin species showing the reproducibility for the 4 replicate injections.

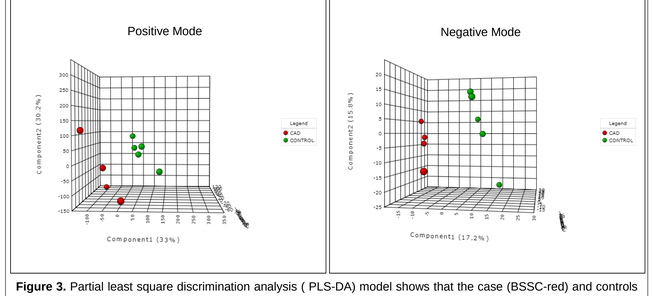

Figure 3. Partial least square discrimination analysis (PLS-DA) model shows that the case (BSSC-red) and controls (BSSN-green) are segregated in two distinct groups in positive and negative mode based on the various lipids species which are the discriminating features between the two groups.

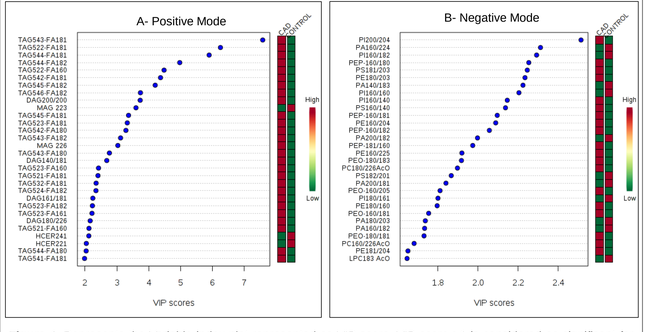

Figure 4. Represents the Variable index plot represented as VIP score. VIP score >1 is considered as significant for the lipid species which are discriminating in PLS-DA analysis. Figure shows the Top 30 predicting features for CAD. Panel A) Positive mode B) Negative mode

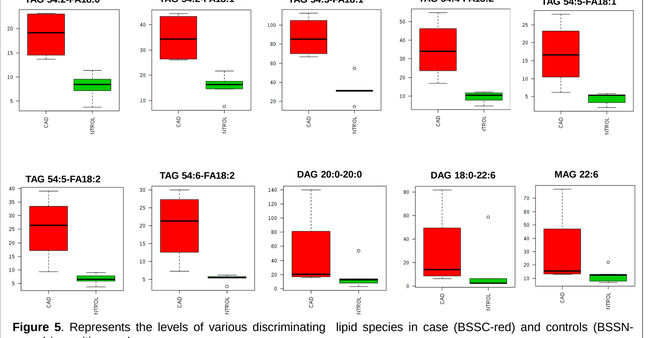

Figure 5. Represents the levels of various discriminating lipid species in case (BSSC-red) and controls (BSSN-green) in positive mode.

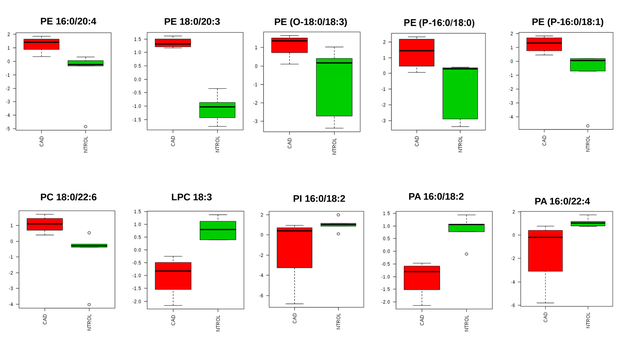

Figure 6. Represents the levels of various discriminating lipid species in case (BSSC-red) and controls (BSSN-green) in negative mode.

CONCLUSIONS

A rapid and economical high throughput technique for the detection and quantification of more than 1000 lipids was developed. A simplified sample preparation, which uses only 10µl of plasma, in combination with gradient LC, and *Scheduled* MRM™ was used. The SCIEX QTRAP® 6500+ LC-MS/MS system was used to develop a robust method where transitions of negative and positive polarity were captured in a single run.
This method led to the identification of several metabolites involved in lipid metabolism, whose levels were significantly different in CAD samples. Total of 92 lipid species representing different classes were found to be altered in CAD patients. Mainly TAGs were found to be upregulated and PEs from phospholipids were found to be down regulated in CAD plasma compared to control plasma samples.
Our study indicates that plasma lipid profiling is a powerful tool for disease research and may contribute to an expanded understanding of the disease progression of CAD.

ACKNOLEGEMENT

This study was supported by Council of Scientific and Industrial Research (BSC-0122).

TRADEMARKS/LICENSING

Document number: RUO-MKT10-7855-A