

Lipidomics Identified Lyso-phosphatidylcholine and Phosphatidylethanolamine as Novel Biomarkers for Diagnosis of Laryngeal Cancer

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Research

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Abstract

Objective

Laryngeal cancer (LaC) remains one of the most common tumors of the respiratory tract with higher incidence in men than in women. The larynx is a small but vital organ on the neck. The dysfunction of the larynx can cause serious health problems such as hoarseness, respiratory distress, and dysphonia. Lipid has been recognized as a crucial role in tumorigenesis. However, the lipid biomarkers is lacking and the lipid molecular pathogenesis of LaC has remained obscure to date. In this study, we aimed to investigate the potential

Methods

To identify new LaC-related lipid biomarkers used for the diagnosis or early diagnosis of LaC and to uncover their molecular characteristics, we conducted serum lipidomics study from LaC patients (n = 29) and normal controls (NC) (n = 36) using nontargeted lipidomics profiling based on ultrahigh-performance liquid chromatography (UHPLC)/Q-TOF 5600 Plus mass spectrometry. Multivariate and univariate statistics analyses were used to discriminate LaC patients from NC.

Results

As expected, a lipid panel including LPC (16:0) and PE (18:0p_20:4) was found to distinguish the LaC patients from healthy individuals with very high diagnosis performance (area under the curve (AUC) = 1.000, sensitivity = 1.000, and specificity = 1.000). In addition, the levels of Cer, CerG1, SM, PC, PC-O, PE, PI, PS, and ChE in the LaC group significantly increased as compared with the NC group. However, the levels of LPC, LPC-O, LPE, LPE-p, and DG in the LaC group significantly decreased when the one was compared with the NC group. Among significantly changed lipid species, lysophospholipids containing a palmitoyl chain or an arachidonic acid acyl chain remarkably decreased and phospholipids including a palmitoyl chain or an arachidonic acid acyl chain increased in the LaC patients.

Conclusion Our results not only indicate that lipidomics is powerful tool to explore abnormal lipid metabolism for the laC, but suggest that lysophospholipids and phospholipids may serve as potential biomarkers for diagnosis of laC.

1. Introduction

Laryngeal cancer is the most common head and neck cancer, causing heavy health care and economic burdens. The Global Burden of Disease Cancer Collaboration currently estimated incidence of LaC is 211 per 1,000, with a 5:1 male to female ratio and approximately 10% of patients in metastatic or terminal phase [1]. Notably, the burden of this malignancy (expressed in years lived with disability (YLDs)) has increased by nearly 25% over the past 30 years [2].

Some LaC patients have been diagnosed at the advanced stage and have an unsatisfactory treatment effect [3]. Early detection of LaC is essential for the treatment of this disease. Although some imaging methods, such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) scan, are commonly used in the screening and detection of LaC, current imaging methods are challenged by issues related to availability of primary healthcare workers who are capable of assessing images. Therefore, there is still an urgent need to identify novel biomarkers for LaC screening or detection.

Lipidomics, focusing on comprehensive profiling of lipids in complex biological matrices, is a powerful tool to identify disease-related lipid biomarkers, and to explore disordered lipid metabolism in the development of diseases. It has been widely applied in many studies, such as diabetes [4, 5], lung cancer [6], liver cancer [7, 8], and so on. Unfortunately, no laryngeal cancer-associated lipidomic study has yet been performed.

The aim of this study is to obtain lipidome profiling data utilizing nontargeted lipidomics method based on UHPLC/Q-TOF mass spectrometry to identify reliable serum biomarkers for the diagnosis of LaC and early-stage LaC and to comprehensively elucidate the abnormal metabolism associated with the onset and development of LaC. Thus, a total of 65 participants were enrolled to discover a novel biomarker panel and test its diagnosis performance and to explore metabolic profiles and abnormal metabolic pathways associated with LaC (Fig. 1).

2. Materials And Methods

2.1 Clinical samples

Serum samples collected from 29 patients diagnosed with laryngeal cancer (LaC) and a cohort of 36 normal controls (NC) were from the Second Affiliated Hospital of Dalian Medical University (Dalian, China). Among these LaC serum samples, 15 cases were in the early stage of laryngeal cancer, i.e. T1NOMO stage. The LaC serum samples were collected prior to surgical resection and stored at -80°C for lipidomics analysis. Eighteen laryngeal cancer tissue (LaCT) and 16 adjacent noncancerous tissue (ANT) samples were also provided by the Second Affiliated Hospital of Dalian Medical University (Dalian, China). Among these LaC tissue samples; 11 cases were in T1NOMO stage. This study was approved by the Second Affiliated Hospital of Dalian Medical University Institutional Ethics Review Board, and all participants provided written informed consent.

2.2 Sample preparation

Frozen serum samples are thawed and homogenized with vortex. A total of 300 μL of cold methanol including 7 lipid internal standards (i.e., PC (19:0/19:0), PE (17:0/17:0), LPC (19:0), SM (d18:1/12:0), TG (15:0/15:0/15:0), Cer (d18:1/17:0), and FFA (16-d3)) was added into 40 μL of each serum sample, followed by a 30-sec vortex. Then, 1 mL of MTBE was added and the mixture was vibrated for 10 min. After that, 300 μL of ultrapure water was added and the extraction mixture was vortexed, and centrifuged

(14,000 rpm at 4 °C) until to form the two-phase system. 400 µL aliquot of the upper organic phase were drawn and dried in a vacuum centrifuge and then stored at – 80 °C until LC-MS analysis. Quality control (QC) samples were produced by pooling equal aliquots from each serum sample and pretreated using the same procedures as that used for the real patient samples. The QC sample was run every 6 real samples in the batch to evaluate the reproducibility of the pretreatment and the LC- MS system.

Tissue sample were weighed. 400 µL of cold methanol including 7 lipid internal standards (i.e., PC (19:0/19:0), PE (17:0/17:0), LPC (19:0), SM (d18:1/12:0), TG (15:0/15:0/15:0), Cer (d18:1/17:0), and FFA (16-d3)) was added, followed by the homogenization at 25 Hz for 2 min. And then 1 mL of MTBE was added and the mixture was vibrated for 15 min. After that, 300 µL of ultrapure water was added and the extraction mixture was vortexed, and centrifuged (14,000 rpm at 4 °C) until to form the two-phase system. 200 µL aliquot of the upper organic phase were drawn and dried in a vacuum centrifuge and then stored at – 80 °C until LC-MS analysis.

Information on lipid internal standards that were spiked in sample matrix prior to lipidom extraction are summarized in Table S1.

2.3 Lipidomics analysis

The lipidome was analyzed using ultra-high-performance liquid chromatography (UHPLC) (Waters, Milford, USA) coupled with a Triple TOF 5600 Plus mass spectrometer (AB SCIEX, USA) system. Prior to the analysis, the lyophilized samples were reconstituted in CH₂Cl₂-MeOH solution (2:1 v/v) and then diluted in a mixed solvent containing ACN-MeOH-H₂O (65:30:5 v/v/v). Subsequently, 5 µL of the diluted samples were separated using a C8 ACQUITY™ column (100 × 2.1 mm, 1.7 µm). The column temperature and elution rate were set at 60 °C and 0.3 mL/min, respectively. The mobile phases A and B, which were ACN:H₂O (6:4 v/v) and IPA: ACN (9:1 v/v), respectively, both contained 10 mM ammonium acetate. The initial elution gradient started with 50% B, maintained for 1.5 min, followed by a linear increase to 85% B at 9.0 min, and then reached 100% B within 0.1 min, held for 1.9 min. At last, it returned to 50% B within 0.1 min and held for 1.9 min for column equilibration. The mass spectrometry signal was scanned from m/z ranges of 200–1250 Dalton in both positive and negative ion modes. The capillary voltages were set at 5.5 kV and – 4.5 kV for positive and negative ion modes, respectively. The interface heater temperature were set at 500°C and 550°C for positive and negative ion modes, respectively.

2.4 Data processing and statistical analysis

The lipids were identified according to previous published paper [9]. The quantitation of the detected lipids was operated by MultiQuant™ 2.1 (AB SCIEX, Concord, Canada) with a mass width of ± 0.05 Da and t_R width of ± 0.25 min. All lipidomics data were normalized by corresponding lipid internal standards.

The supervised partial least-squares discriminant analysis (PLS-DA) was performed by SIMCA-P software (13.0 version, Umetrics Umeå, Sweden), using Pareto scaling mode, which divided each variable by the square root of the standard deviation to suppress noise interference. Nonparametric test in Wilcoxon, Mann–Whitney test mode, was implemented for the comparisons between the LaC and NC groups to

identify significantly differential lipids ($p < 0.01$ & $FDR < 0.05$) and heatmap was produced by the open-source software MultiExperiment Viewer (MeV, version 4.9.0). Receiver operating characteristic (ROC) curves of a binary logistic regression were performed using SPSS software version 19 (SPSS, Inc.). The bar graph of the significantly changed lipid classes was made by the GraphPad Prism software (Version 6.0).

3. Results

3.1 Clinical characteristics of patients with laryngeal cancer and normal controls

The detailed characteristics of the LaC and NC groups are provided in Table 1. Twenty-nine patients who underwent resection of laryngeal cancer were staged for laryngeal cancer based on the 8th edition of the AJCC Cancer Staging Manual. In this study, T1N0M0 stage of laryngeal was also taken into consideration for diagnosing the disease at an early stage. The age and sex between the LaC group and the NC group are matched as much as possible.

3.2 Serum lipidomics identify the entire lipidom alternations between patients with LaC and the NC

Lipidomics profiling was performed to comparatively analyse the serum samples collected from the patients with LaC ($n = 29$) and the NC ($n = 36$) groups. In this study, 390 lipids were identified by exact mass-to-charge ratio (M/Z), retention time (t_R), and/or characteristic fragments. Among these identified lipids, 17 common lipid (sub)classes, containing FA, LPC, LPE, PC, PE, Cer, SM, DG, and TG, etc., were identified (Fig. S1A). The QC sample were inserted into the analytical batch after 6 real samples to monitor the lipidomics data quality. In Fig. S1, relative standard deviations (RSD) of 47% and 87% of the detected lipids were less than 10% and 20%, respectively. The percentage of the detected lipids with RSD less than 30% could reach at 98%, which confirmed the analytical reliability of the LC-MS-based lipidomics method used to collect the lipidom data. The detected lipids with RSD less than 30% were used for the Follow-up statistical analysis.

A supervised PLS-DA model was made based on those identified lipids from serum samples. In Fig. 2A, we could find that the LaC group was apparently distinguished from the NC group. Subsequently, 200 time of permutations were operated to evaluate whether the PLS-DA model is over-fitting. In Fig. 2B, $R^2 = (0.0, 0.359)$ and $Q^2 = (0.0, -0.456)$ shown that this model is stable. These results implied that substantial lipidom alternations occurred underlying the onset and development of LaC. Sixty-two lipids with $VIP > 1.0$ were identified as important variables to contribute the classifications.

3.3 Defining of potential lipidomics biomarkers for LaC

To explore significantly differential lipid species between the LaC group and the NC group, a univariate analysis (non-parameter test) was performed based on the lipidomics data from the LaC and NC groups.

The levels of 204 lipids were observed to be significantly different between patients with LaC and the NC ($p < 0.01$ and false discovery rate (FDR) < 0.05). The details of the differential lipids are shown in Table S2. Finally, 43 of these lipids exhibited $p < 0.01$, FDR < 0.05 and VIP > 1.0 in the two comparisons (Fig. 2C). In addition, 43 differential lipids were subjected to the heatmap visualization to obtain a pattern overview of lipidomics alterations with the development of LaC in a clinical setting (Fig. 2D). Subsequently, a binary logistic regression analysis and an optimized algorithm of the forward stepwise (Wald) method were used to construct the best model using these 43 potential lipid biomarkers. Finally, the combination of LPC (16:0) and PE (18:0p_20:4) (Fig. 3A,C) was defined as the ideal biomarker panel to distinguish patients with LaC from normal controls.

The diagnostic performance of this lipid panel was notably high, such as AUC value of 1.000, sensitivity value of 1.000, and specificity value of 1.000 in the discrimination of LaC from NC in the serum sample, respectively (Fig. 4A). Furthermore, the serum metabolite panel had a perfect performance in identifying the LaC_{T1N0M0} at early-stage LaC from the NC group, such as AUC, sensitivity, and specificity values of 1.000, 1.000, and 1.000, respectively (Fig. 4B).

3.4 Characteristics of lipid (sub)classes between the LaC and NC groups

lipid profiling among two studied groups was further investigated at the level of a given lipid (sub)class. To this end, the summed content of all individual lipids within a given (sub)class was compared between the LaC and NC groups by Student T- test ($p < 0.05$). The results shown that the relative levels of Cer, CerG1, SM, PC, PC-O, PE, PI, PS, and ChE in the LaC group significantly accumulated as compared with the NC group. The levels of LPC, LPC-O, LPE, LPE-p, and DG in the LaC group significantly decreased when the one was compared with the NC group (Table 2).

Another interesting finding was that a large number of PLs (PC, PE, and PI lipids) containing a palmitoyl chain and/or an arachidonic acid acyl chain were significantly increased in LaC vs NC, and LPCs with a palmitoyl chain and/or an arachidonic acid acyl chain were significantly decreased in LaC vs NC (Fig. 5).

4. Discussion

In this study, most of clinical characteristics between group LaC and NC showed no significant difference. Their observable phenotype differences obviously could not be explained by the clinical findings. Lipids are essential in cellular functions as they are the vital components of the membrane structure, crucial regulators in signal transduction and energy storage. Abnormalities in lipid metabolism have been increasingly regarded as a landmark of tumor cells and are involved in many human diseases. We performed herein a comprehensive lipidome analysis of larynx tumors in patients with LaC and the controls. To the best of our knowledge, this is the first report on a systemic evaluation of tumor lipid metabolism in patients with LaC.

Our lipidomic study exhibited four primary findings. First, a lipid biomarker panel was identified to detect LaC patients from healthy individuals, with high diagnosis performance. Second, LaC caused substantial changes in lipid compositions. These changes were largely driven by accumulations in sphingolipids including Cer and SM lipids, PLs including PC, PE and PI lipids and ether PC and PE lipids. Third, LysoPLs containing LPC and LPE were significantly reduced in LaC as compared to NC. Fourth, we found that PLs containing palmitic acid or arachidonic acid residues were significantly accumulated and lysoPLs containing palmitic acid or arachidonic acid residues were significantly decreased, in LaC as compared to NC. These findings suggest that LaC may cause the enhancement of sphingolipid and PL synthesis, and inflammatory reactions.

Currently, the identification of novel potential serum biomarkers for the detection of LaC remains a vital goal, particularly for the diagnosis of early-stage LaC. However, only a few biomarker candidates have been translated to clinical applications due to the limited study cohorts or diagnostic performance. In the present study, we employed the nontargeted lipidomics method to screen biomarkers. Some of the biomarkers may be among the unidentified lipids, but for clinical application, they should be identified. Therefore, we did our best to identify as many lipids as possible (Table S2). After systematic selection using multivariate and univariate statistical analyses, a biomarker panel consisting of LPC (16:0) and PE (18:0p_20:4) was identified. The serum biomarker panel separated LaC from the NC with very high performance. Moreover, this lipid panel effectively discriminated patients with LaC_{T1N0M0} from the NC, highlighting the early diagnostic potential of this lipid biomarker panel.

The relative levels of most Cer and SM lipids were significantly increased in patients with LaC compared with healthy controls. Ceramide is bioactive lipids of the sphingolipid pathway and play essential roles in cell signaling. Ceramide has been shown to be involved in stress-related cellular responses and apoptosis [10, 11]. The imbalance in ceramide will greatly affect the physicochemical properties of cell, leading to cellular dysfunction. It has been demonstrated that ceramide metabolism is altered in numerous cancers characterized by an elevation of the Cer profile in tumor tissue and cancer cells [7, 12, 13]. We speculated that the significant increase in the level of the Cer lipid class in LaC could have resulted from the elevated expression of the enzymes responsible for the synthesis of ceramide. It was reported that ceramide synthase in a salvage pathway was highly activated in several different tumors, such as human colon cancer [14], human non-small-cell lung cancer [15]. In vivo, Cer can be also generated by the hydrolysis of SM through the actions of sphingomyelinases. Upregulating the endogenous Cer level is regarded to be a novel therapeutic target for the anticancer intervention strategy [16]. In all, we hypothesized that reducing Cer biosynthesis or preventing from converting SM to Cer could inhibit LaC progression.

It is well known that PL is one of the most important components of a mammalian membrane bilayer. PC is the most predominant constituent of PLs in the cellular membrane. It has been demonstrated that PC metabolism is altered in the onset and development of many cancers, characterized by an elevation of PC [17–19]. We deduced that the significant increase in PC may be due to an imbalance between PC and PE. Increased PC and an imbalance between PC and PE have been reported to be associated with obesity and NAFLD [20, 21], both of which are also associated with LaC occurrence. In addition, PC and LPC mutually

convert, upregulating PC level may come from LPC conversion. This point can be supported by the significantly decreased level of LPC in LaC patients. Altogether, disordered PC lipid metabolism is closely associated with the development of LaC.

PLs containing PUFA, especially arachidonic acid residues, were significantly increased in LaC in this study. Arachidonic acid is a major PUFA in mammals. Long-chain acyl-coenzyme A synthetase 4 (ACSL4), belonging to the ACSL family, shows a preferential use of arachidonic acid as its substrate and plays a role in the remodeling of AA-containing phospholipids by incorporating free AA. In consideration of the significant increase of AA-residue-enriched PLs (e.g., PC(16:0_20:4), PE(16:0_20:4), PE(16:0p_20:4), PE(18:0p_20:4), PI(16:0_20:4), etc.), and the level of AA, so-called FA (20:4) significantly decreased in LaC serum, we speculated that ACSL4 may be activated and thereby prompt PLs accumulation, which associated with a greater degree of oncogenic changes of the neoplastic cell of LaC characterized by the great abundance of mitochondria. Of course, further investigation should be performed to explore our findings.

5. Conclusion

In summary, using nontargeted lipidomics method based on UHPLC-HRMS, we successfully identified a lipid biomarker panel (including LPC(16:0) and PE(18:0p_20:4)) that can effectively diagnose LaC from their cohort of healthy controls. Similar, this panel shows ultrahigh performance in detection of the early-stage LaC from the healthy volunteers. To our best knowledge, this study demonstrates for the first time the systemic alteration in the lipid composition between LaC vs NC. Cer, SM, and AA-enriched PLs showing close association with LaC, may be potential biomarkers and act as potential targets for LaC. Considering the given small sample size, to guarantee the rationality of our findings, further studies based on large-scale clinical samples and on the expression of related lipidenzymes will be required.

Abbreviations

LaC, laryngeal cancer; NC, normal control; PL, phospholipid; AA, arachidonic acid; UHPLC-HRMS, ultrahigh-performance liquid chromatography–high-resolution mass spectrometry; QC, quality control; RSD, relative standard deviation; Cer, ceramide; CerG1, glucosylceramide; CerG2, galactosylceramide; SM, sphingomyelin; ChE, cholesterol ester; DG, diacylglycerol; TG, triacylglycerol; FA, fatty acid; OAHFA, (O-acyl)-1-hydroxy fatty acid; LPC, lyso-phosphatidylcholine; LPC-O, LPC with alkyl substituents; LPE, lyso-phosphatidylethanolamine; LPE-p, LPE with alkenyl substituents; PC, phosphatidylcholine; PC-O, PC with alkyl and alkenyl substituents; PE, phosphatidylethanolamine; PE-p, PE with alkenyl substituents; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SL, sphingolipid; PLS-DA, partial least squares- discrimination analysis; LysoPL, lysophospholipid; PUFA, polyunsaturated FA.

Declarations

Funding

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Declaration of Competing Interest

The authors declare no competing financial interest.

Contributions

JZ. W. and B. Y. conceived and designed the project; B. Y. performed the experiments and analyzed the data, collected the clinical samples; B. Y. and JZ .W wrote the manuscript, with input from all the authors.

References

1. C. Global Burden of Disease. Cancer C, Fitzmaurice D, Abate N, Abbasi H, Abbastabar F, Abd-Allah, et al., Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2017: A Systematic Analysis for the Global Burden of Disease Study, *JAMA Oncol*, 5 (2019) 1749–1768.
2. < james2018.pdf >.
3. Smith MM, Abrol A, Gardner GM. Assessing delays in laryngeal cancer treatment. *Laryngoscope*. 2016;126:1612–5.
4. Razquin C, Toledo E, Clish CB, Ruiz-Canela M, Dennis C, Corella D, Papandreou C, Ros E, Estruch R, Guasch-Ferre M, Gomez-Gracia E, Fito M, Yu E, Lapetra J, Wang D, Romaguera D, Liang L, Alonso-Gomez A, Deik A, Bullo M, Serra-Majem L, Salas-Salvado J, Hu FB. M.A. Martinez-Gonzalez, Plasma Lipidomic Profiling and Risk of Type 2 Diabetes in the PREDIMED Trial. *Diabetes Care*. 2018;41:2617–24.
5. Lu Y, Wang Y, Zou L, Liang X, Ong CN, Tavintharan S, Yuan JM, Koh WP, Pan A. Serum Lipids in Association With Type 2 Diabetes Risk and Prevalence in a Chinese Population. *J Clin Endocrinol Metab*. 2018;103:671–80.
6. Klupczynska A, Plewa S, Kasprzyk M, Dyszkiewicz W, Kokot ZJ, Matysiak J. Serum lipidome screening in patients with stage I non-small cell lung cancer. *Clin Exp Med*. 2019;19:505–13.
7. Simon J, Ouro A, Ala-Ibanibo L, Presa N, Delgado TC. M.L. Martinez-Chantar, Sphingolipids in Non-Alcoholic Fatty Liver Disease and Hepatocellular Carcinoma: Ceramide Turnover, *Int J Mol Sci*, 21 (2019).
8. Hall Z, Chiarugi D, Charidemou E, Leslie J, Scott E, Pellegrinet L, Allison M, Mocciaro G, Anstee QM, Evan GI, Hoare M, Vidal-Puig A, Oakley F, Vacca M, Griffin JL, Lipid remodelling in hepatocyte

- proliferation and hepatocellular carcinoma, *Hepatology*, (2020).
9. Xuan Q, Zheng F, Yu D, Ouyang Y, Zhao X, Hu C, Xu G. Rapid lipidomic profiling based on ultra-high performance liquid chromatography-mass spectrometry and its application in diabetic retinopathy. *Anal Bioanal Chem*. 2020;412:3585–94.
 10. < perry1996.pdf >.
 11. Parveen F, Bender D, Law SH, Mishra VK, Chen CC, Ke LY, Role of Ceramidases in Sphingolipid Metabolism and Human Diseases, *Cells*, 8 (2019).
 12. Aslan M, Afsar E, Kirimlioglu E, Ceker T, Yilmaz C. Antiproliferative Effects of Thymoquinone in MCF-7 Breast and HepG2 Liver Cancer Cells: Possible Role of Ceramide and ER Stress, *Nutr Cancer*, (2020) 1–13.
 13. Schwalm S, Erhardt M, Romer I, Pfeilschifter J, Zangemeister-Wittke U, Huwiler A. Ceramide Kinase Is Upregulated in Metastatic Breast Cancer Cells and Contributes to Migration and Invasion by Activation of PI 3-Kinase and Akt, *Int J Mol Sci*, 21 (2020).
 14. Medatwal N, Ansari MN, Kumar S, Pal S, Jha SK, Verma P, Rana K, Dasgupta U, Bajaj A. Hydrogel-mediated delivery of celastrol and doxorubicin induces a synergistic effect on tumor regression via upregulation of ceramides. *Nanoscale*. 2020;12:18463–75.
 15. Suzuki M, Cao K, Kato S, Mizutani N, Tanaka K, Arima C, Tai MC, Nakatani N, Yanagisawa K, Takeuchi T, Shi H, Mizutani Y, Niimi A, Taniguchi T, Fukui T, Yokoi K, Wakahara K, Hasegawa Y, Mizutani Y, Iwaki S, Fujii S, Satou A, Tamiya-Koizumi K, Murate T, Kyogashima M, Tomida S, Takahashi T. CERS6 required for cell migration and metastasis in lung cancer, *J Cell Mol Med*, (2020).
 16. Ogretmen B. Sphingolipid metabolism in cancer signalling and therapy. *Nat Rev Cancer*. 2018;18:33–50.
 17. < 6732.full.pdf >.
 18. < 10.1016@S0006-291 × 0200920-8.pdf >.
 19. Iorio E, Ricci A, Bagnoli M, Pisanu ME, Castellano G, Di Vito M, Venturini E, Glunde K, Bhujwalla ZM, Mezzanzanica D, Canevari S, Podo F. Activation of phosphatidylcholine cycle enzymes in human epithelial ovarian cancer cells. *Cancer Res*. 2010;70:2126–35.
 20. Arendt BM, Ma DW, Simons B, Noureldin SA, Therapondos G, Guindi M, Sherman M, Allard JP. Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine to phosphatidylethanolamine. *Appl Physiol Nutr Metab*. 2013;38:334–40.
 21. Weir JM, Wong G, Barlow CK, Greeve MA, Kowalczyk A, Almasy L, Comuzzie AG, Mahaney MC, Jowett JB, Shaw J, Curran JE, Blangero J, Meikle PJ. Plasma lipid profiling in a large population-based cohort. *J Lipid Res*. 2013;54:2898–908.

Tables

Due to technical limitations, table 1 and table 2 are only available as a download in the Supplemental Files section.

Figures

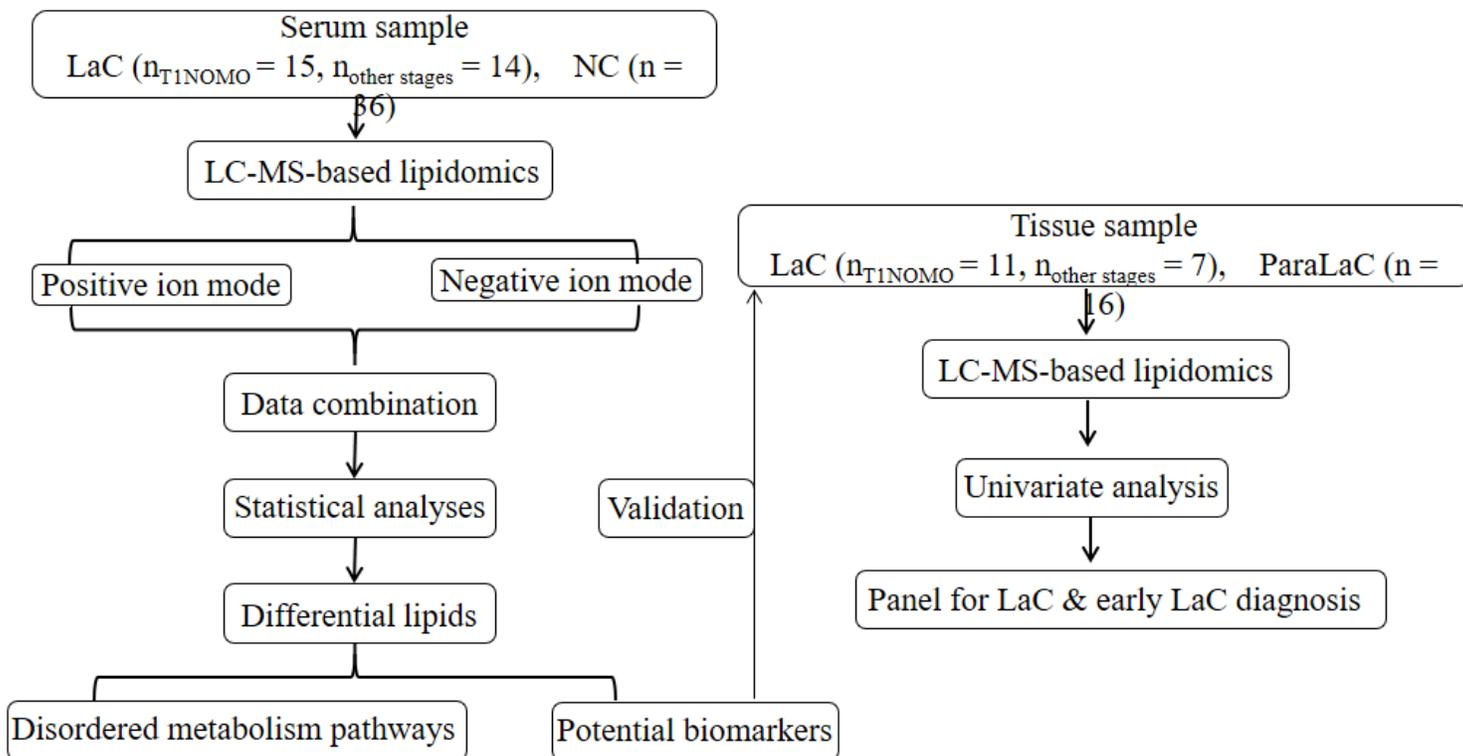


Figure 1

Workflow of the study design.

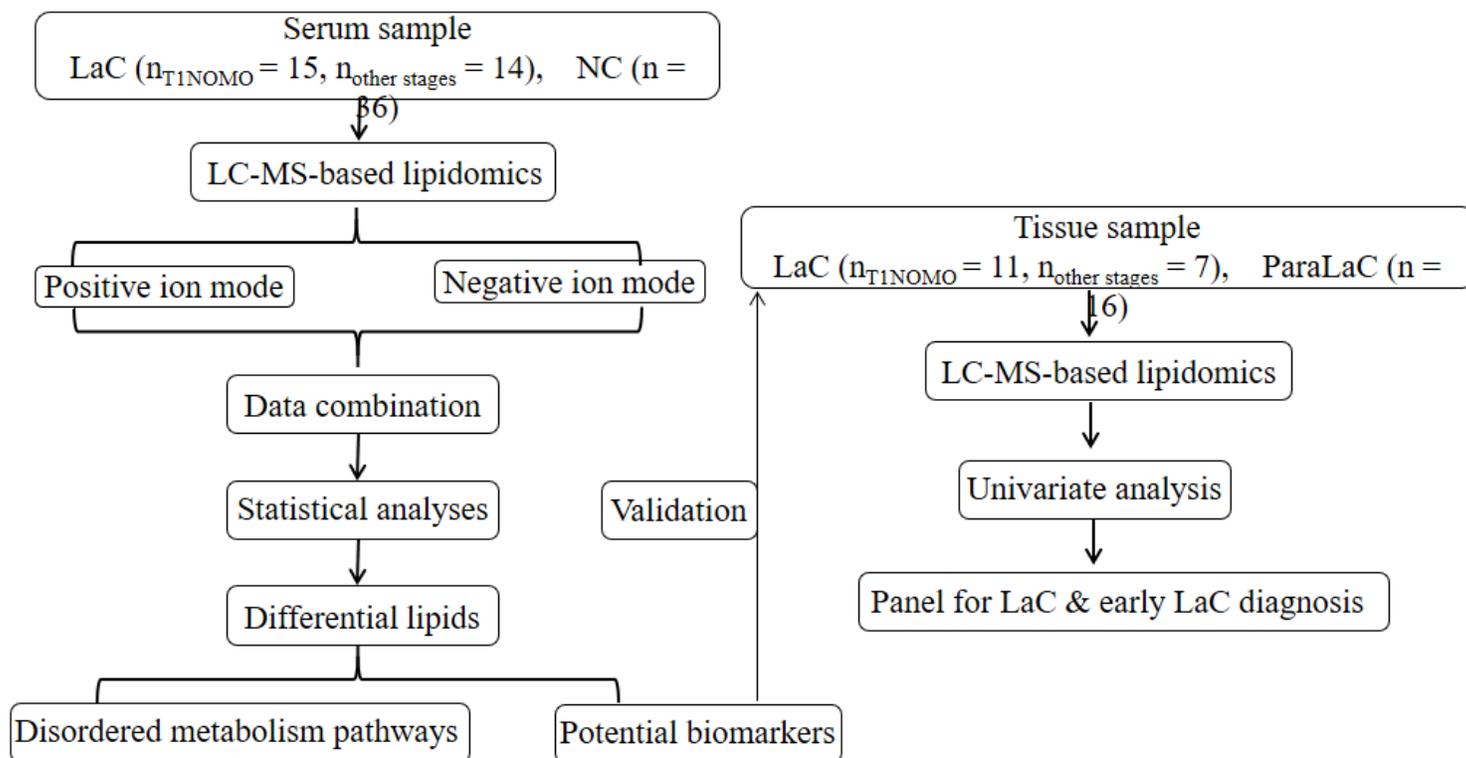


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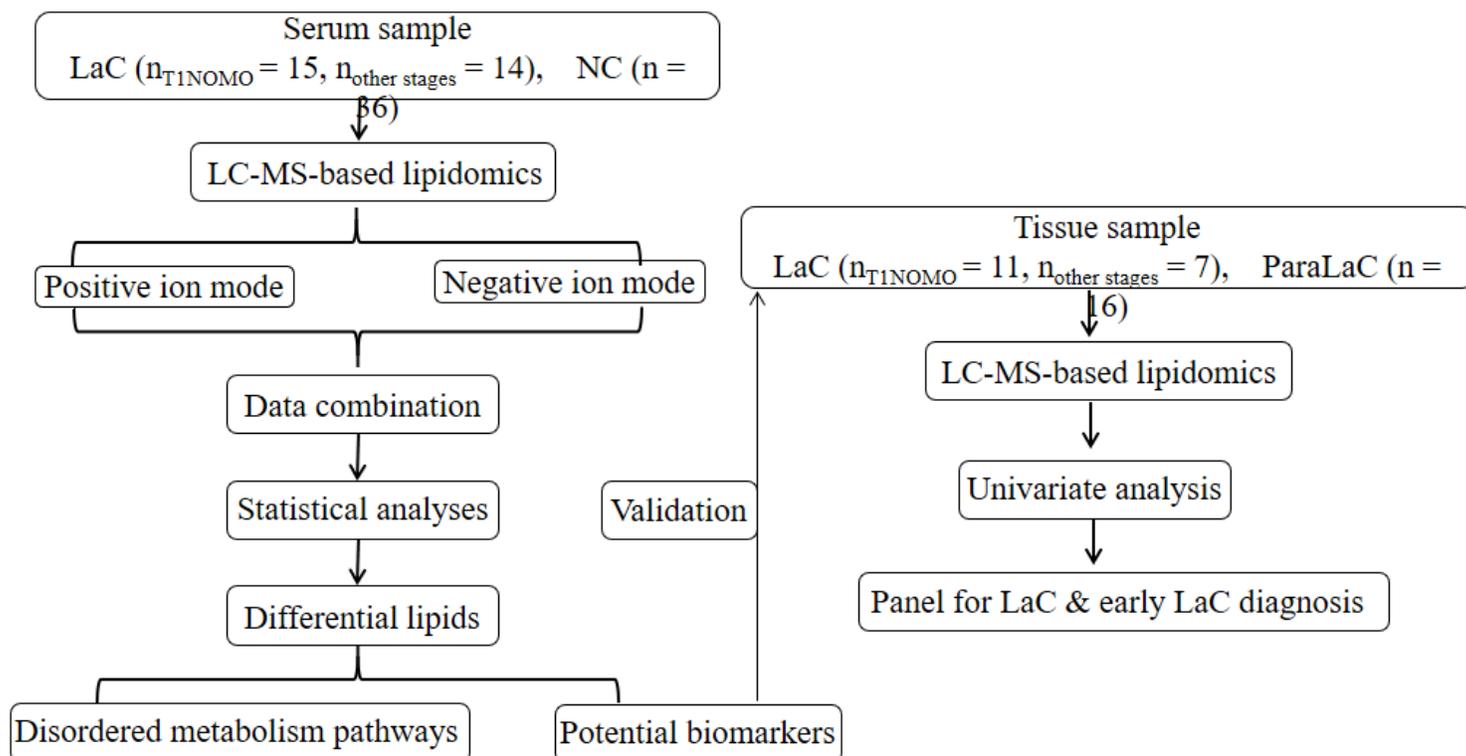


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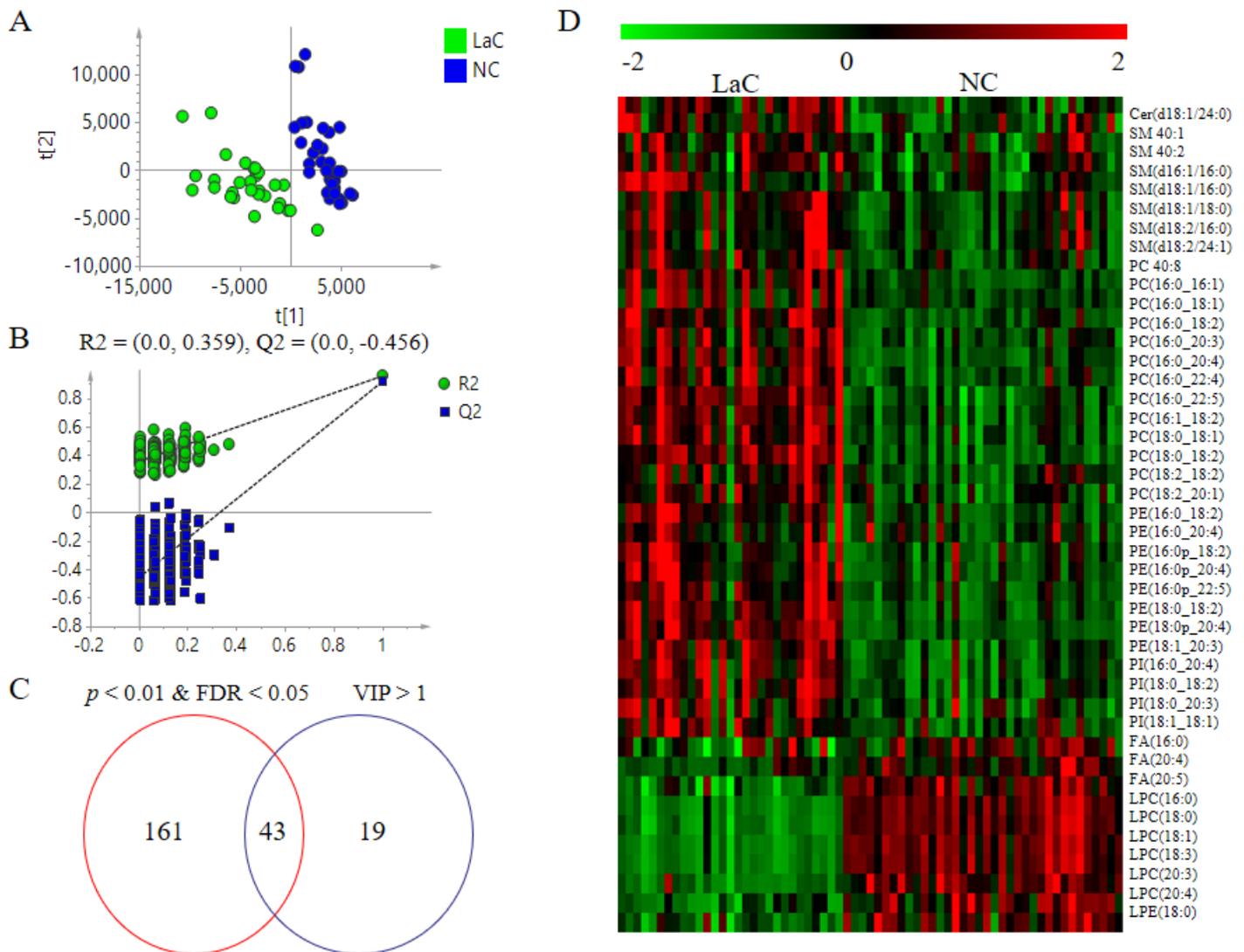


Figure 2

(A) Score plots of PLS-DA between LaC and NC for lipidomics data from serum samples. (B) Cross validation of PLS-DA model between LaC and NC for lipidomics analyses. (C) Venn diagram displays the differential lipids when the LaC group was compared with the NC group in serum samples. (D) Heatmap overview of the 43 differential lipids whose level changes can distinguish patients with LaC from normal controls

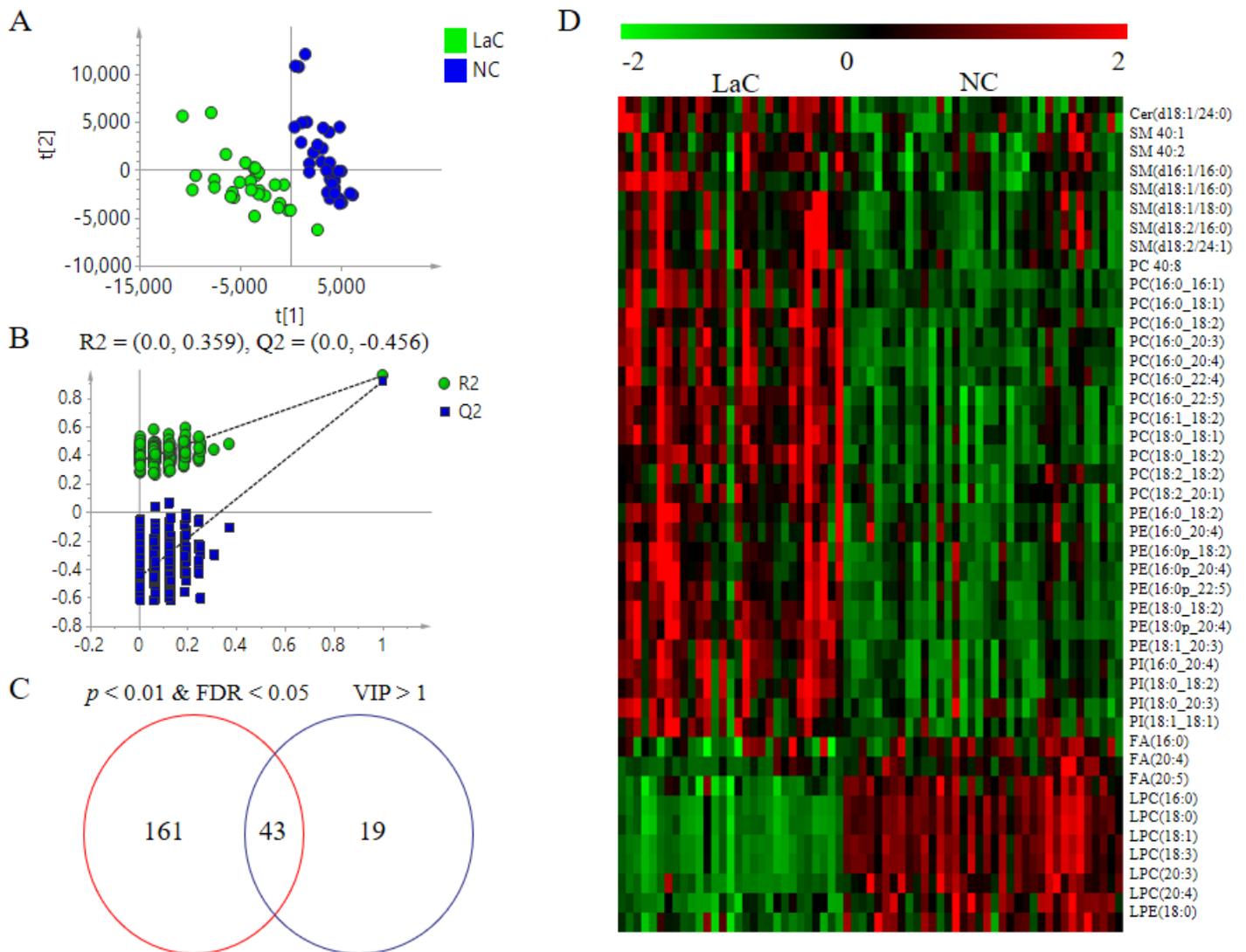


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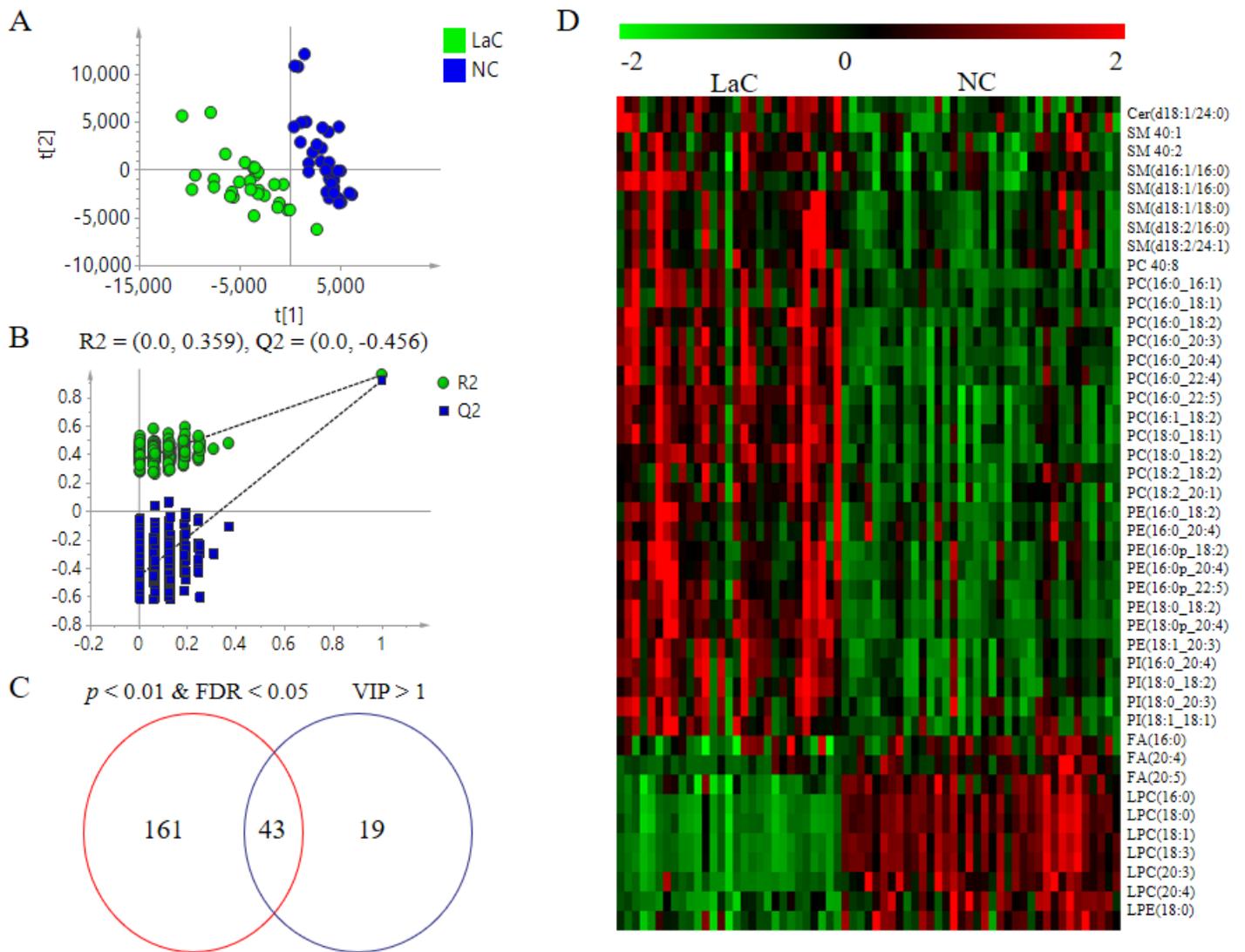


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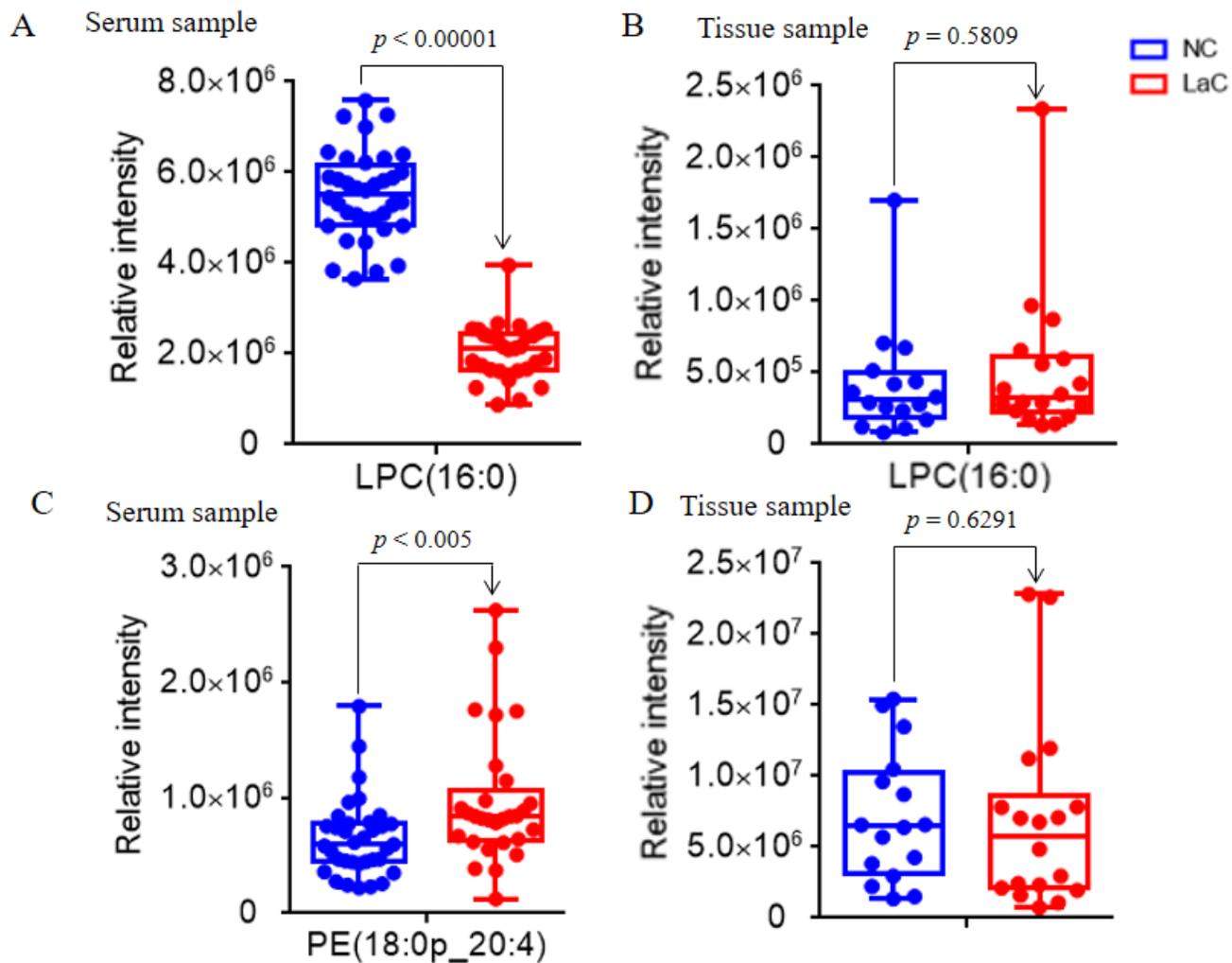


Figure 3

(A) and (C) Serum relative intensity of defined potential biomarkers of LPC(16:0) and PE(18:0p_20:4), respectively. (B) and (D) Tissue relative intensity of defined potential biomarkers of LPC(16:0) and PE(18:0p_20:4), respectively.

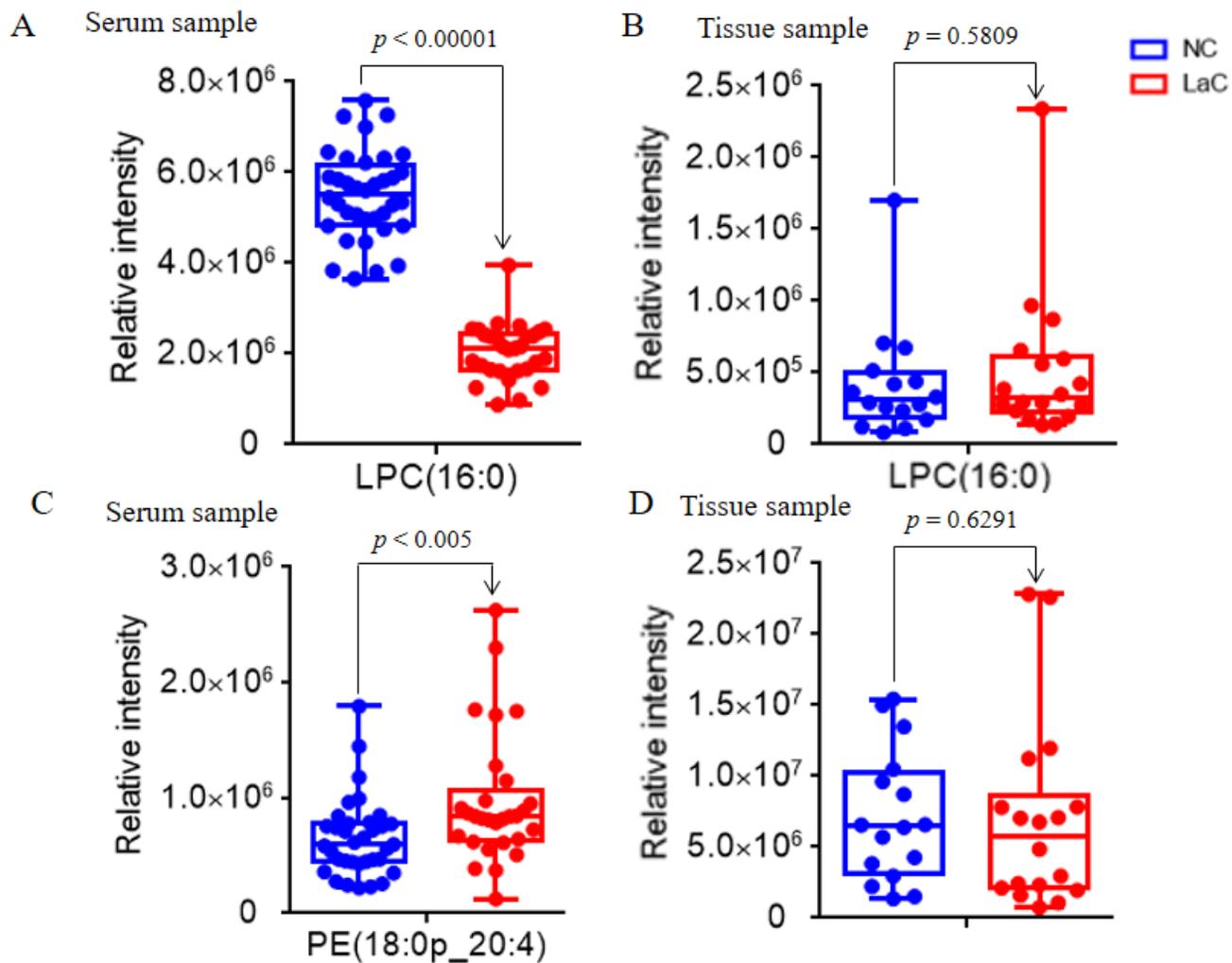


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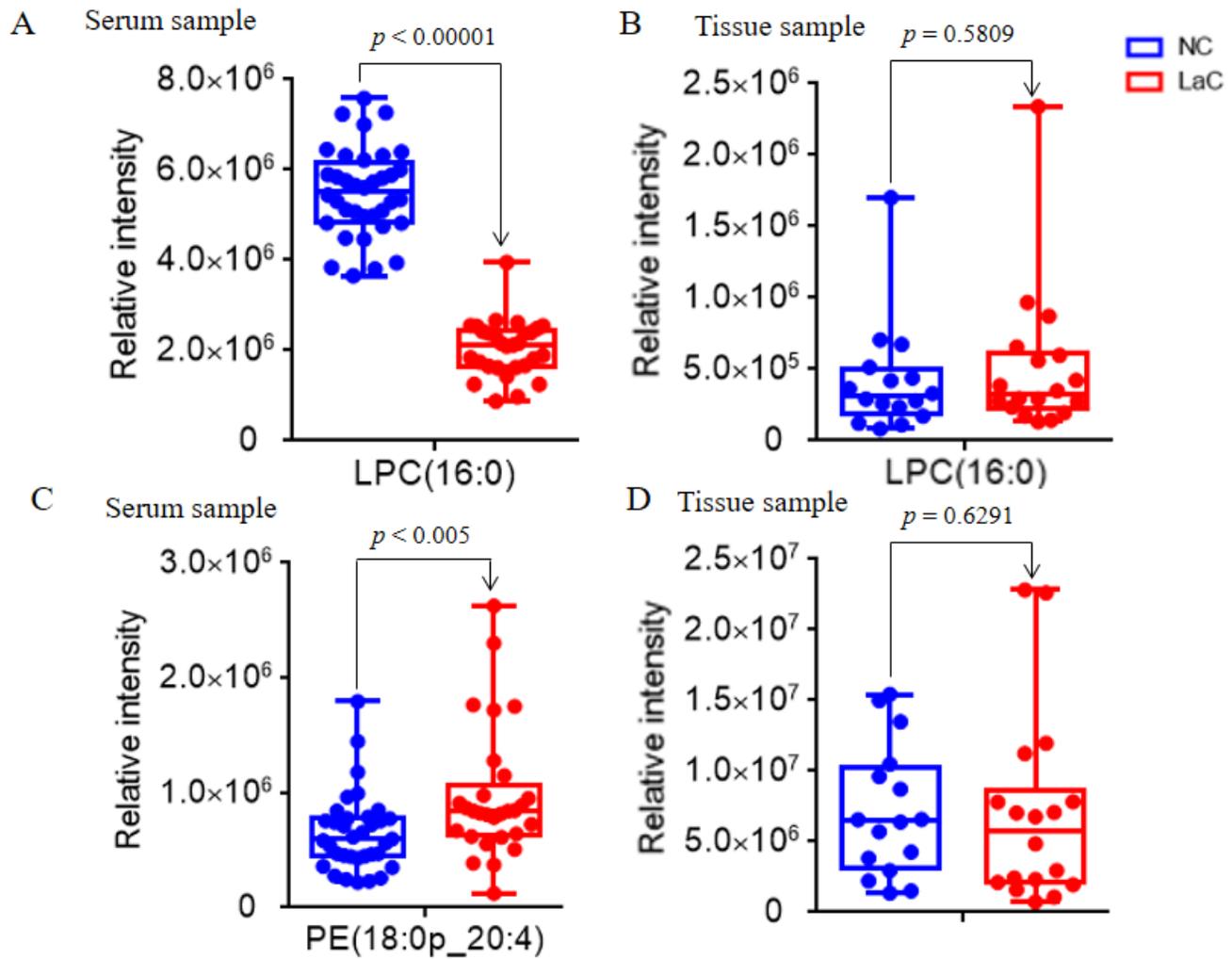


Figure 3

(A) and (C) Serum relative intensity of defined potential biomarkers of LPC(16:0) and PE(18:0p_20:4), respectively. (B) and (D) Tissue relative intensity of defined potential biomarkers of LPC(16:0) and PE(18:0p_20:4), respectively.

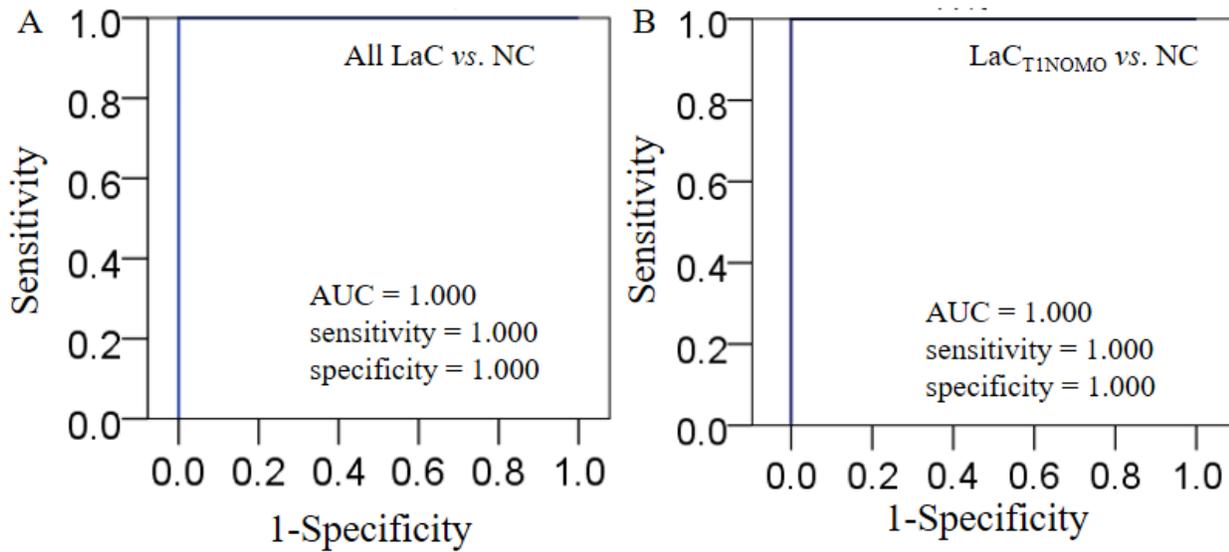


Figure 4

⊠A⊠ Characterization of ROC curve of lipid panel in the serum samples collected from the LaC group and NC group. ⊠B⊠ Characterization of ROC curve of lipid panel in the serum samples collected from the LaC_{T1NOMO} group and NC group. AUC: Area under curve; Lipid panel: LPC(16:0) and PE(18:0p_20:4).

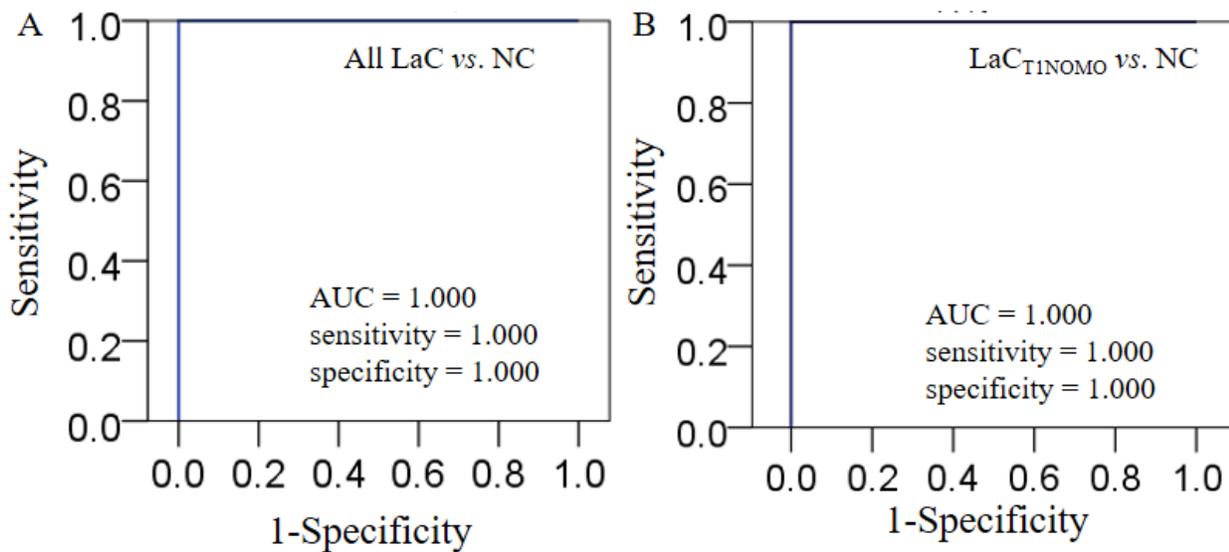


Figure 4

⊠A⊠ Characterization of ROC curve of lipid panel in the serum samples collected from the LaC group and NC group. ⊠B⊠ Characterization of ROC curve of lipid panel in the serum samples collected from the LaC_{T1NOMO} group and NC group. AUC: Area under curve; Lipid panel: LPC(16:0) and PE(18:0p_20:4).

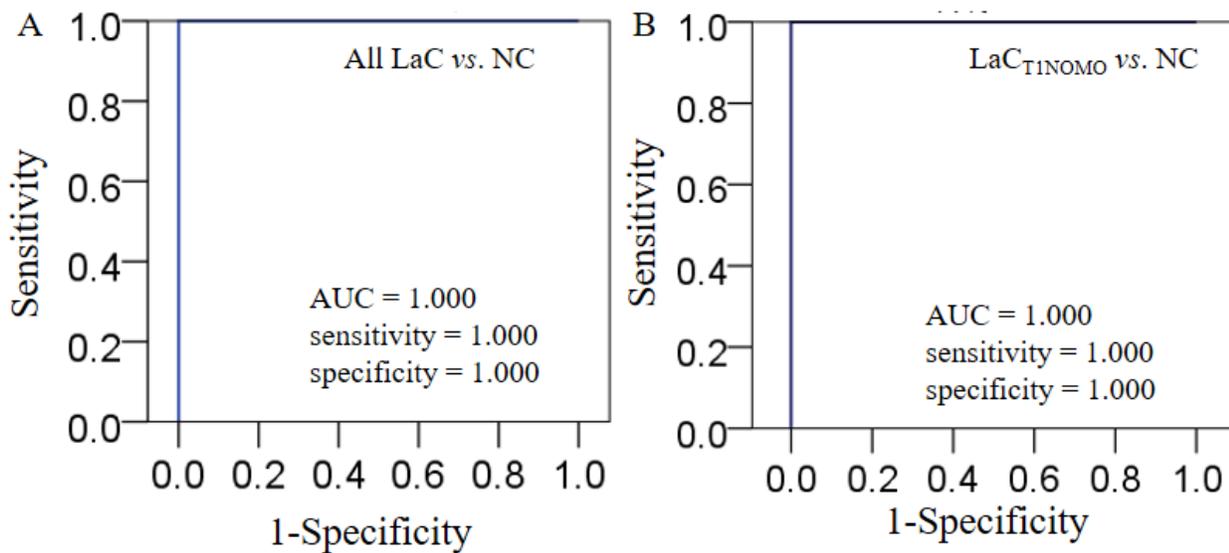


Figure 4

A Characterization of ROC curve of lipid panel in the serum samples collected from the LaC group and NC group. **B** Characterization of ROC curve of lipid panel in the serum samples collected from the LaC_{T1NOMO} group and NC group. AUC: Area under curve; Lipid panel: LPC(16:0) and PE(18:0p_{20:4}).

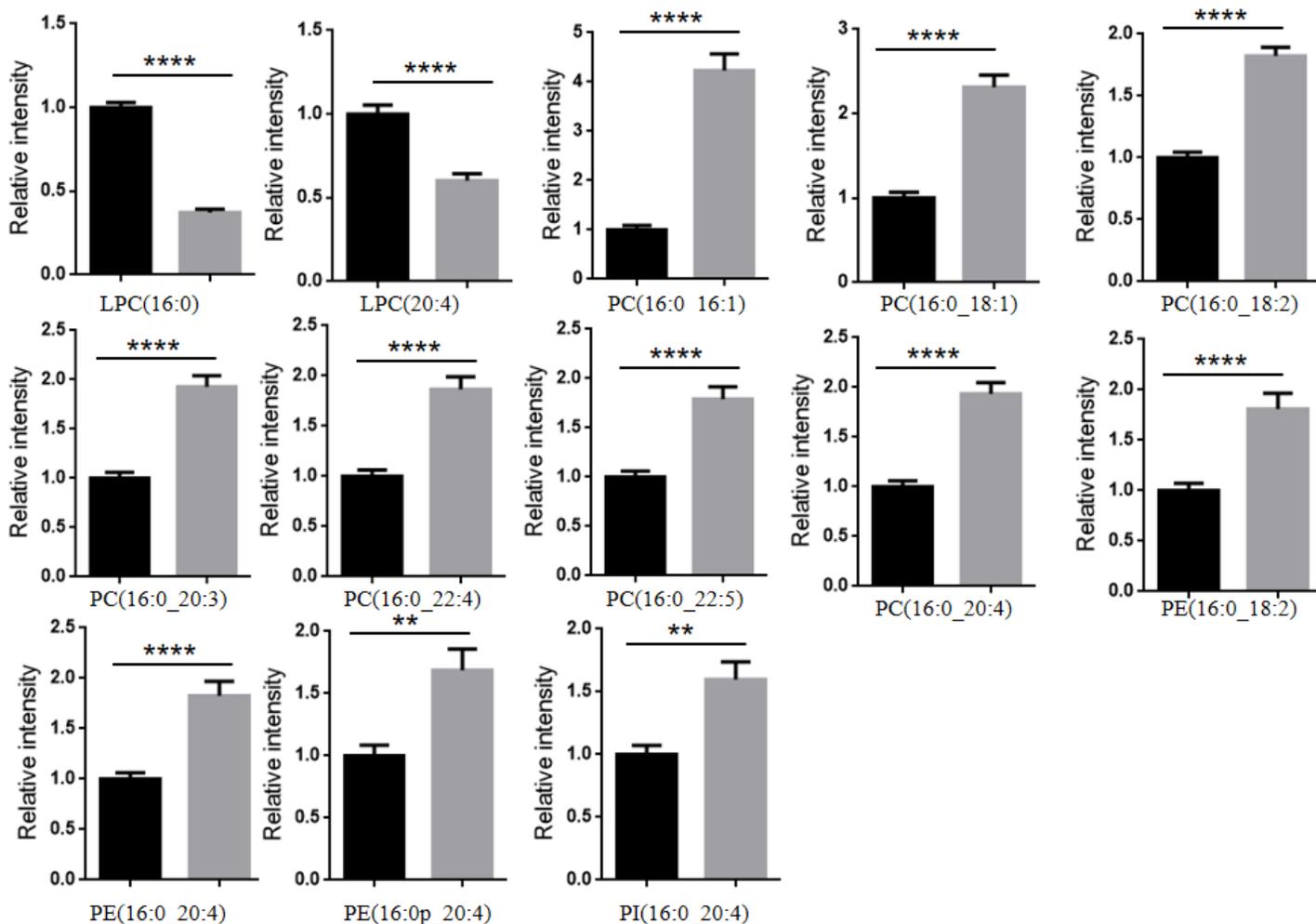


Figure 5

LPLs containing a palmitoyl chain or an arachidonic acid acyl chain were significantly decreased and PLs containing a palmitoyl chain or an arachidonic acid acyl chain were mostly significantly increased in LaC vs. NC in serum samples. **** $p < 0.00001$, ** $p < 0.001$.

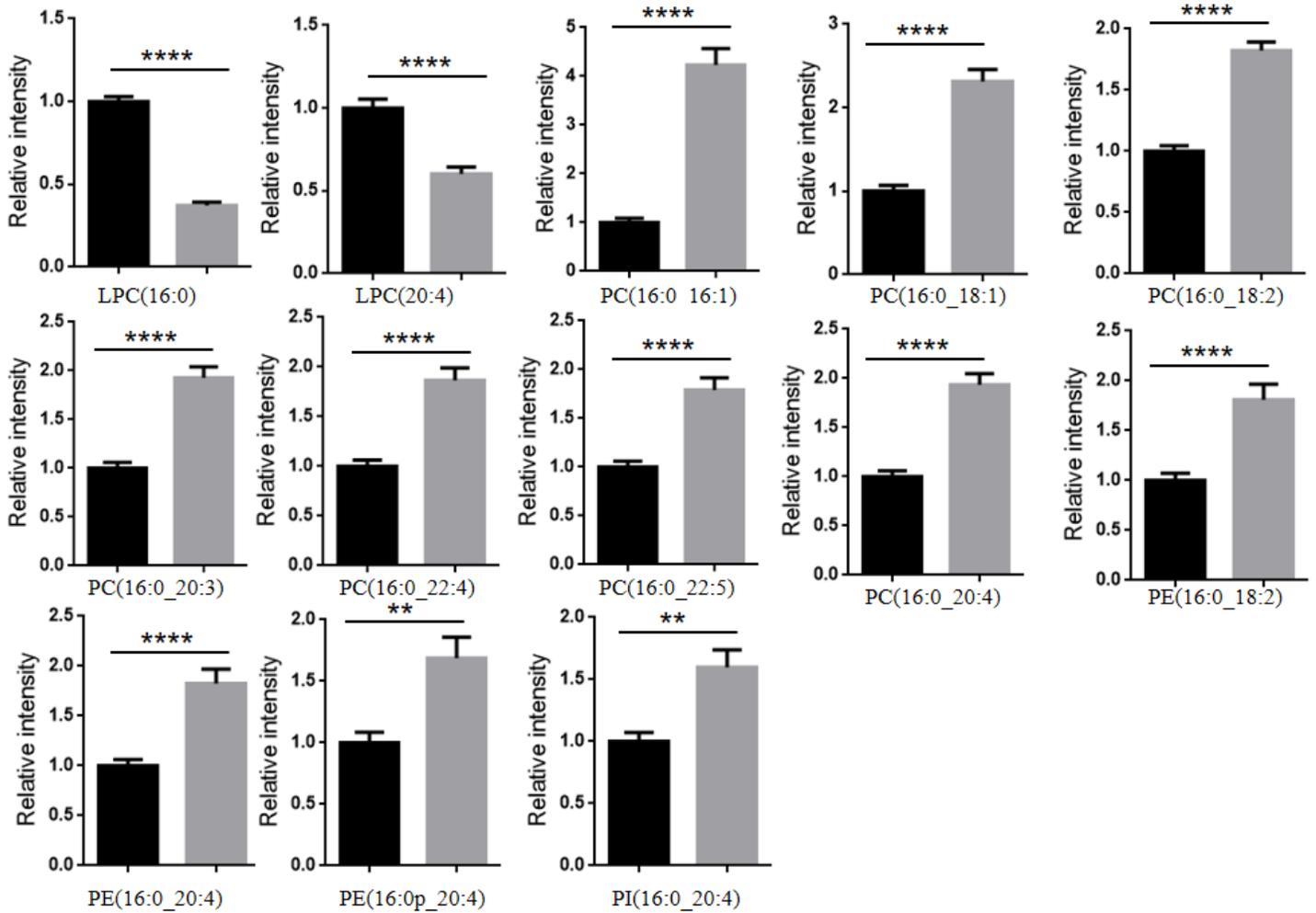


Figure 5

LPLs containing a palmitoyl chain or an arachidonic acid acyl chain were significantly decreased and PLs containing a palmitoyl chain or an arachidonic acid acyl chain were mostly significantly increased in LaC vs. NC in serum samples. **** $p < 0.00001$, ** $p < 0.001$.

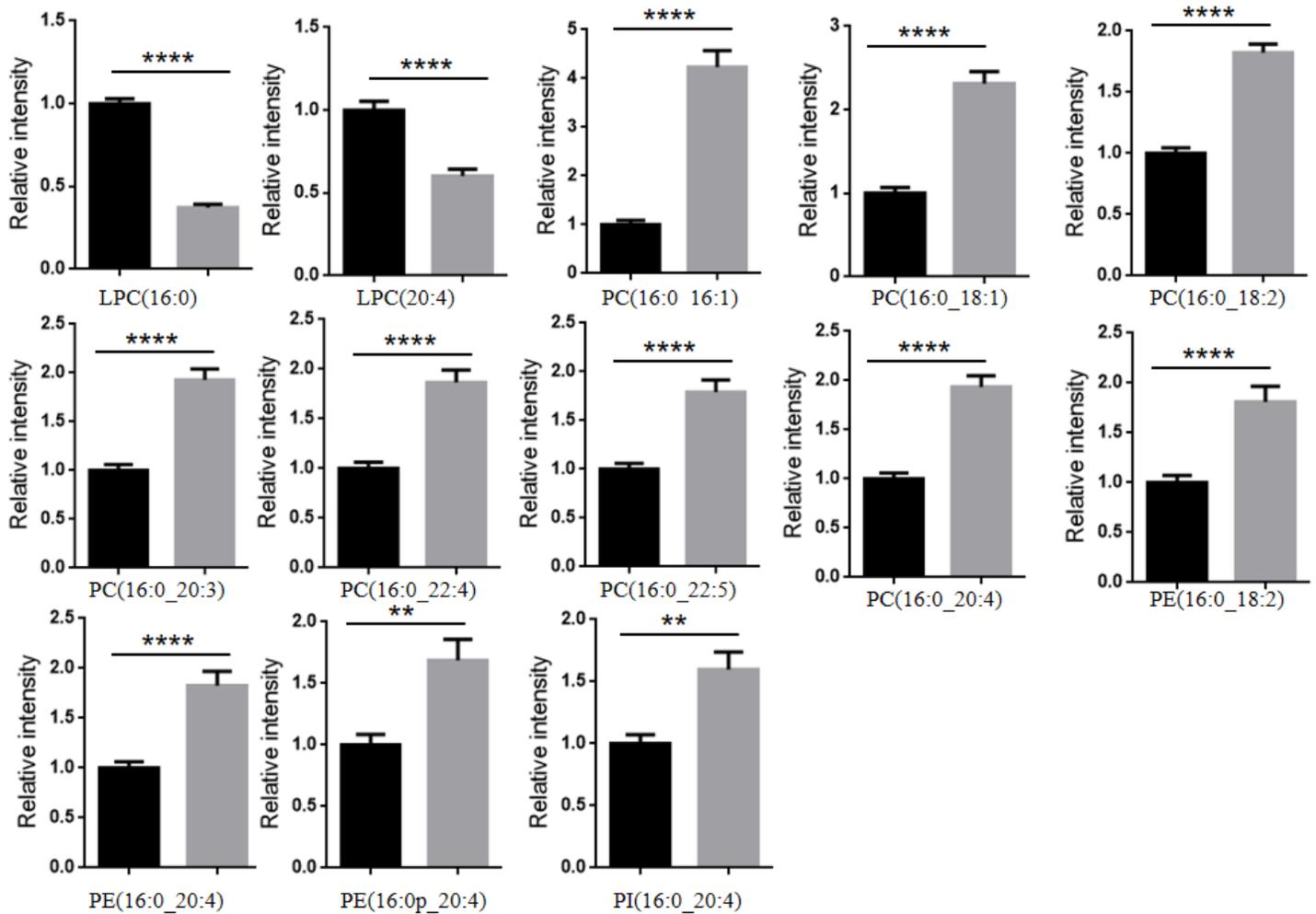


Figure 5

LPLs containing a palmitoyl chain or an arachidonic acid acyl chain were significantly decreased and PLs containing a palmitoyl chain or an arachidonic acid acyl chain were mostly significantly increased in LaC vs. NC in serum samples. **** p < 0.00001, ** p < 0.001.

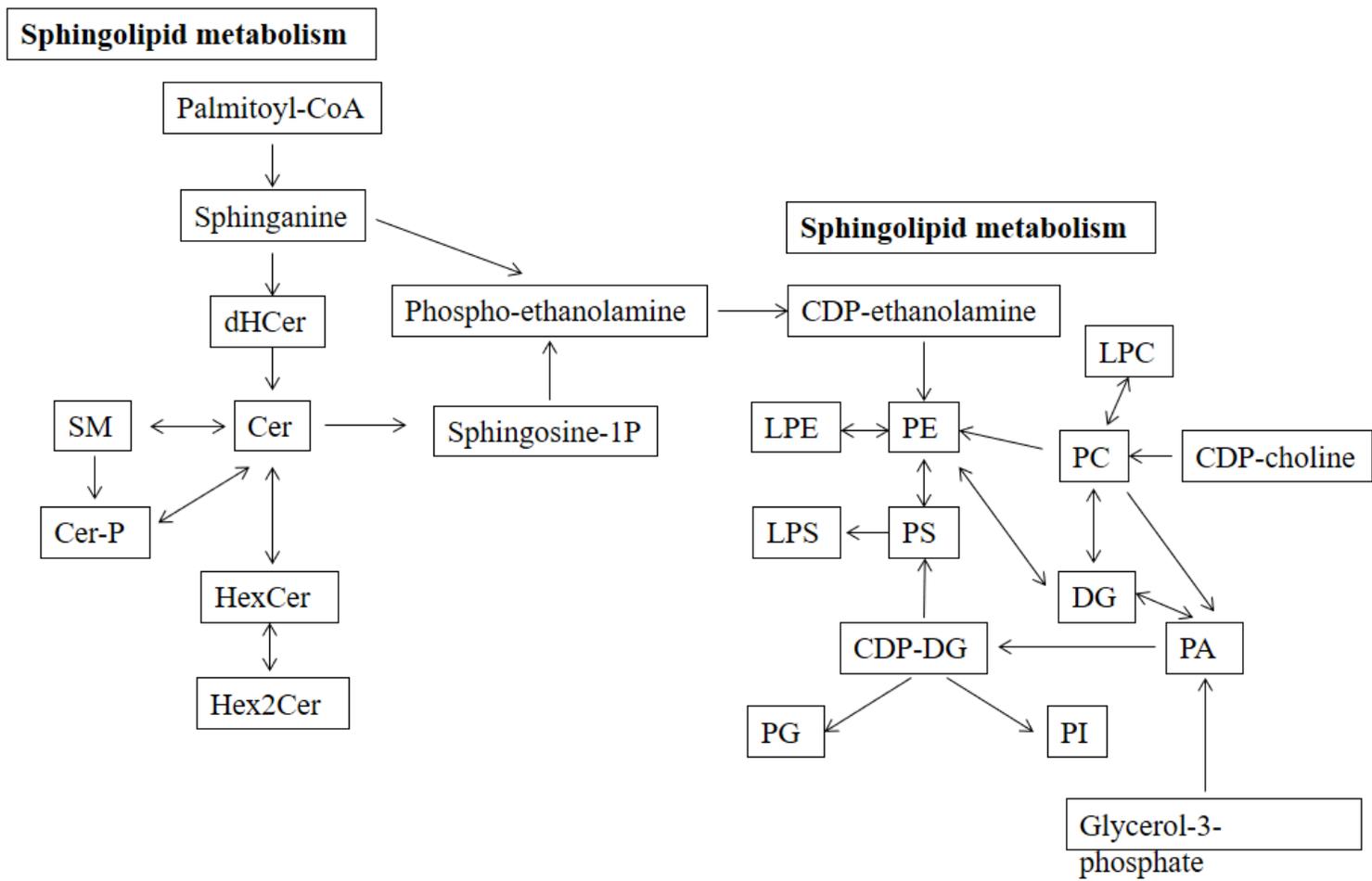


Figure 6

Sphingolipid metabolism

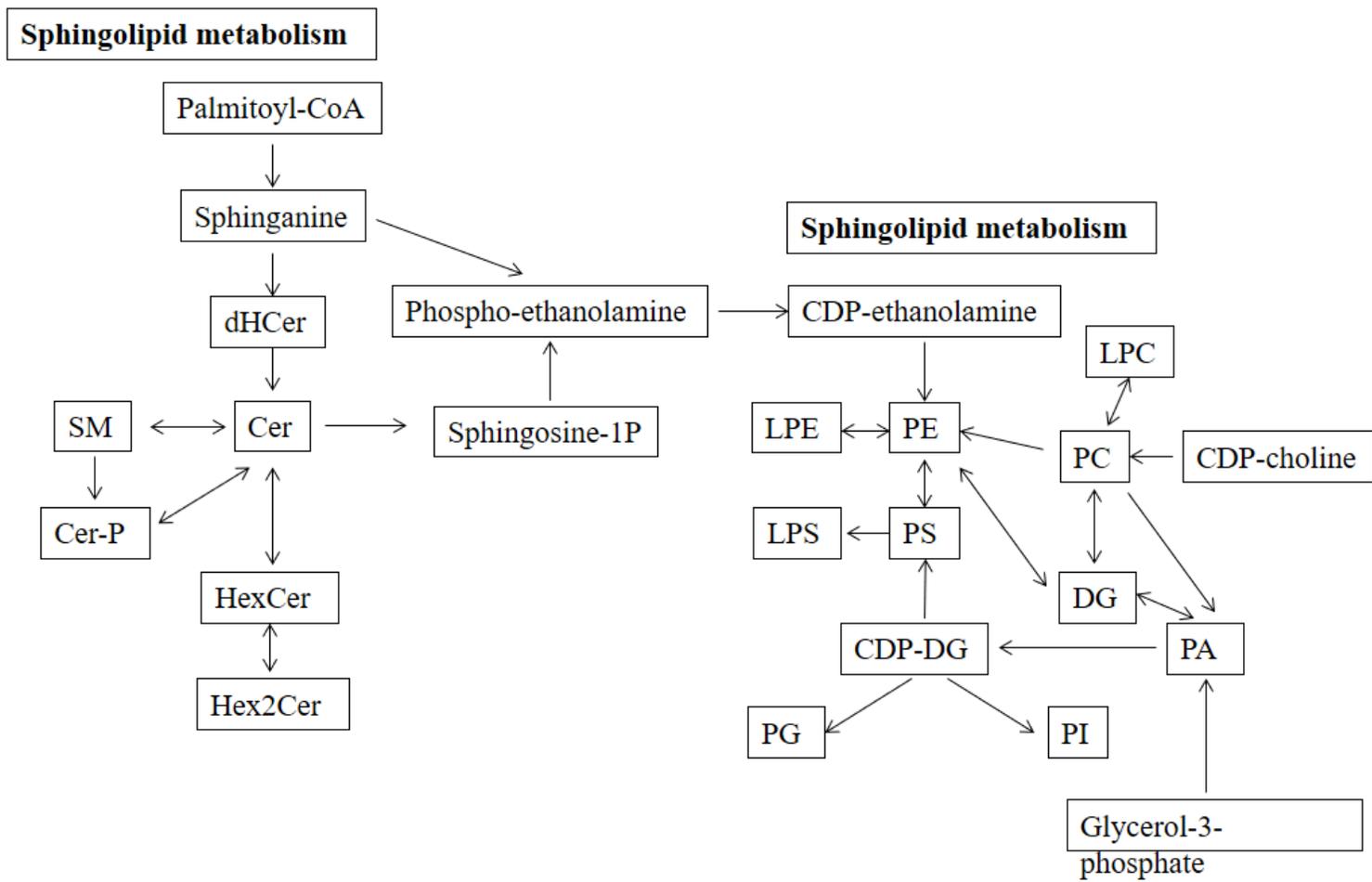


Figure 6

Sphingolipid metabolism

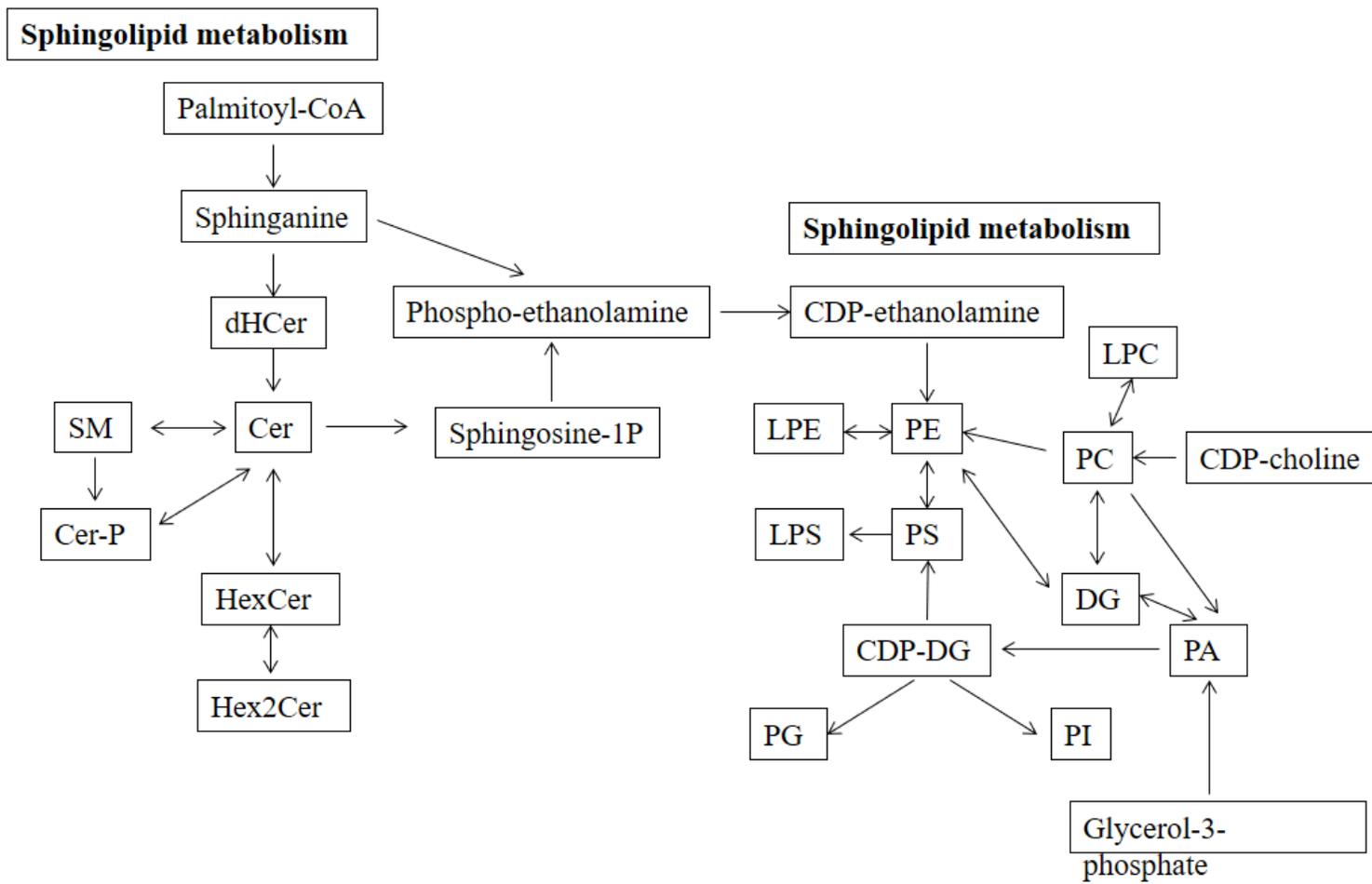


Figure 6

Sphingolipid metabolism

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