Serum Metabolomic Signatures Associated with Disease Severity in Allergic Rhinitis

Shaobing Xie
Xiangya Hospital Central South University
Hua Zhang
Xiangya Hospital Central South University
Zhihai Xie
Xiangya Hospital Central South University
Yongzhen Liu
Xiangya Hospital Central South University
Kelei Gao
Xiangya Hospital Central South University
Junyi Zhang
Xiangya Hospital Central South University
Ruohao Fan
Xiangya Hospital Central South University
Shumin Xie
Xiangya Hospital Central South University
Fengjun Wang
Xiangya Hospital Central South University
Weihong Jiang (✉ jiangwh68@126.com)
Xiangya Hospital Central South University

Research

Keywords: Allergic rhinitis, Metabolomics, Metabolites, Severity

Posted Date: July 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-40854/v1

Abstract

Background: Allergic rhinitis (AR) is a global healthcare problem with obscure pathogenesis, and few studies have evaluated the association between AR and metabolomics. The aim of this study was to identify differences in serum metabolomics profiling of AR patients compared to healthy controls and to explore novel biomarkers reflecting disease severity.

Methods: Serum samples were collected form 29 healthy controls and 72 AR patients, including 30 mild AR patients (MAR) and 42 moderate to severe AR patients (MSAR). Metabolomic profiling was performed by ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) in serum samples. Orthogonal partial least square-discriminate analysis (OPLS-DA) was applied to assess the differences between AR patients and controls and for subgroups based on disease severity.

Results: These analysis results successfully revealed distinct metabolite signatures which distinguished MAR patients and MSAR patients from health controls. In addition, MSAR patients also could be discriminated from MAR patients basing on their metabolic fingerprints. Most observed metabolite changes were related to pathways associated with glycine, serine and threonine metabolism, pyrimidine metabolism, sphingolipid metabolism, arginine and proline metabolism and fatty acid metabolism. Among these metabolites from dysregulated metabolic pathways, levels of sarcosine, sphingosine-1-phosphate (S1P), cytidine and linoleic acid significantly correlated with AR total nasal symptom score (TNSS) and visual analogue scale (VAS).

Conclusions: MSAR patients have a distinctive serum metabolomics profile compared to MAR and health controls. These results suggest that metabolomic profiling may provide novel insights into pathophysiological mechanisms of AR and contribute to its evaluation of disease severity.

Background

Allergic rhinitis (AR) is an IgE-mediated immunologic disease characterized by mucus hypersecretion and airway hyperresponsiveness with characteristic nasal symptoms such as sneezing, rhinorrhea and nasal blockage [1-2]. Epidemiological studies have shown that AR affects at least half a billion people worldwide, and more than half of them suffer from moderate to severe AR [3]. In China, the prevalence of AR even rises to 34.3% of the general population, and rates still continue to increase [4-5]. Although without life threatening, AR exhibits negative influence on people's quality of life and their work production, and brings about a high medical cost on both the individual and society. Currently, AR is subdivided into intermittent AR and persistent AR according to the allergic rhinitis and its impact on asthma (ARIA) classification [6], and persistent AR is further grouped into mild AR (MAR) and moderate to severe AR (MSAR) based on the severity scale. The immunological underpinnings and their associations with disease severity, have been the hot topic of significant research, but remain poorly clarified.

Previous publications have reported that AR is very heterogeneous with a wide degree of severity; meanwhile, there is no novel objective indication or biological marker that is specific for its disease

activity [7-8]. Current monitoring the severity of this clinical disorder relies primarily on subjective clinical symptom score which is relatively insensitive, particularly in children [9-10]. In most AR patients, they often do not recognize how severe their symptoms are because of progressive tolerance to these symptoms. Furthermore, self-reported symptoms used by physicians to guide treatment and evaluate the therapeutic effect are likely imprecise. Potentially, this also can lead to growing costs of healthcare and wasted resources [7]. In addition, clinical research in AR is hampered because of a lack of sensitive biological measures of disease severity. Therefore, identification of biomarkers of disease severity or response to therapy is urgently needed to improve patient management and the accelerate drug development in AR.

Metabolomics, a branch of omics science that systematically analyzes the concentration profiles of low molecular weight endogenous metabolites generated by living systems, is a promising approach to identify new biomarkers and novel metabolic pathways for several diseases, simultaneously provide new insights into underlying pathophysiological mechanisms [11-12]. Recently, several studies have employed metabolomics technologies to explore the metabolic changes in asthma, pneumonia and chronic obstructive pulmonary disease, and successfully identified some potential biomarkers and key metabolic pathways [13-15]. However, no previous study has focused on metabolite profiles and metabolic pathway changes in the serum of AR patients, especially regarding the disease severity.

Therefore, the primary aim of this study was to explore metabolic profiling of AR patients and determine the relationship between the metabolite changes and clinical severity of AR, thus to provide new insights into the complex pathophysiological mechanisms and monitor disease activity and therapeutic effect. In this study, ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) was performed to investigate metabolomic profiling in serum samples of MSAR, in comparison with MAR and healthy control. In addition, linear regression analysis was performed to evaluate the correlation between metabolites and disease severity.

Methods

Participants and settings

This is a prospective study with 101 participants recruited between June 2018 and January 2019. All participants were divided into three groups: MAR (n=30), MSAR (n=42) and health control (n=29). The diagnosis of AR was done on the basis of the medical history and allergic symptoms (sneezing, rhinorrhea, nasal congestion and nasal itching) for at least 2 years, positive skin test results (a mean wheal diameter >3 mm) and positive specific IgE (>0.35 IU/ml). Patients with persistent AR were categorized into MAR and MSAR according to the allergic rhinitis and its impact on asthma (ARIA) criteria [6]. Exclusion criteria included current smoking, other allergic diseases, systemic steroid treatment, inflammatory or septic diseases, autoimmune diseases, age<18 years, pregnant condition, immunotherapy, and use of anti-allergic drugs during the 1 month that preceded the study. The total IgE levels, special IgE for house dust mite (HDM) levels, blood eosinophil count and demographic information

of the study subjects were collected, including gender, age, body mass index (BMI), the duration of disease duration. All participants scored their symptoms by using the widely accepted Total Nasal Symptom Score (TNSS) and visual analogue scale (VAS) which were described by previous study [7, 16]. The TNSS is the sum of 4 individual symptom scores for sneezing, rhinorrhea, nasal congestion, and nasal itching , and each symptom score was regarded on a 4-point scale from 0 to 3 (0=no symptom, 1=minimal, well-tolerated symptoms, 2=bothersome but tolerated symptoms, 3=severe and hard to tolerate symptoms). In addition, the global disease severity over the last week was evaluated by a VAS (0-100 mm: where 0 is no symptom and 100 is the maximum severity). The detailed clinical information of the recruited participants in the three groups was described in Table 1.

Serum sample collection and preparation

Serum samples from AR patients and healthy volunteers were collected with serum separator tubes without anticoagulation or coagulant before breakfast, and serum separator tubes were stored for 1 hour at room temperature. All blood samples were centrifuged at 4 °C (3000 rpm for 10 min), then, we collected the supernatants and stored them at -80 °C in equal aliquots for subsequent detection and analysis. The serum samples were prepared for UHPLC-MS analysis by mixing 100 μL of serum sample with 300 uL methanol containing internal standard (L-2-Chlorophenylalanine, 2 μg/mL). After 30 s vortex, the samples were sonicated for 10 min in ice-water bath. Then the samples were incubated at -40 ℃ for 1 h and centrifuged at 12000 rpm for 15 min at 4 ℃. 100 μL of supernatant was transferred to a fresh glass vial for UHPLC-MS analysis [16]. The quality control (QC) sample was prepared by mixing an equal aliquot of the supernatants from all of the samples, and used to evaluate the reproducibility and reliability of the UHPLC-MS analytical system as described by previous study [17].

UHPLC-MS analysis

The UHPLC separation was carried out using a 1290 Infinity series UHPLC System (Waters Corporation, Milford, MA, USA), equipped with a UPLC BEH Amide column (2.1mm×100 mm, 1.7 μm). The mobile phase consisted of 25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water（pH = 9.75）(A) and acetonitrile (B). The analysis was conducted with elution gradient as follows: 0~0.5 min, 95% B; 0.5~7.0 min, 95%~65% B; 7.0~8.0 min, 65%~40% B; 8.0~9.0 min, 40% B; 9.0~9.1 min, 40%~95% B; 9.1~12.0 min, 95% B. The column temperature was 25℃. The auto-sampler temperature was 4 ℃, and the injection volume was 2 μL (positive) or 2 μL (negative), respectively. The Triple TOF 6600 mass spectrometry (AB Sciex, Boston, MA, USA) was used for its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Analyst TF 1.7, AB Sciex, Framingham, MA, USA) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. In each cycle, the most intensive 12 precursor ions with intensity above 100 were chosen for MS/MS at collision energy (CE) of 30 eV. The cycle time was 0.56 s. Electrospray ionization (ESI) source conditions were set as following: Gas 1 as 60 psi, Gas 2 as 60 psi, Curtain Gas as 35 psi, Source Temperature as 600 ℃,

Declustering potential as 60 V, Ion Spray Voltage Floating (ISVF) as 5000 V or -4000 V in positive or negative modes, respectively.

Data processing and analysis

MS raw data (.wiff) files were converted to the mzXML format by Proteo Wizard, and processed by R package XCMS V3.2. The process includes peak deconvolution, alignment and integration. Minfrac and cut off are set as 0.5 and 0.3 respectively as before [18]. In-house MS2 database was applied for metabolites identification [19]. The resultant data was exported to SIMCA (Version 14.1, Umetrics, Umea, Sweden) for multivariate analysis. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was performed to find potential biomarkers that contributed to the metabolic difference between the groups [20]. The quality of models was validated by R2Ycum (goodness of fit) and Q2cum (goodness of prediction). Meanwhile, the 7-fold cross-validation and 200 permutation tests were conducted to reduce the risk of over-fitting and possibilities of false-positive findings. Metabolites contributing were selected according to the variable importance for project (VIP) values (VIP >1.0) and P-values (P<0.05) [21]. To determine the performance of the identified combination, receiver operating characteristics (ROC) analysis was conducted, and area under the curve (AUC) was calculated to assess the sensitivity and specificity. In order to gain insight into the underlying metabolic mechanisms associated with AR and its severity, the metabolic pathway was analyzed in both ion modes using MetaboAnalyst 3.0.

Statistical analysis

Continuous and categorical variables are described as mean ± Standard deviation (SD) and number (%) respectively. Kolmogorov-Smirnov test was performed to assess the normality of the distribution in continuous variables. One-way analysis of variance (ANOVA) or Student's t test was used, and non-parametric Mann-Whitney test was performed when the variables distributed non-normally. Categorical variables were compared between three groups using Chisquare test. To explore the correlation between levels of metabolites and severity of AR, Spearman's correlation analysis was conducted. Differences were considered as significant when P<0.05. All above statistical analyses were carried out using SPSS statistics software version19.0 (IBM, Chicago, IL, USA).

Results

Baseline characteristics of all participants

The main characteristics and clinical information of the participants were shown in the Table 1. No statistically significant difference was observed in gender, age, BMI, and disease duration among three groups. In comparison with control group and MAR group, MSAR group showed higher levels of serum total IgE, special IgE for HDM, blood eosinophil counts, TNSS and VAS (all P < 0.001).

Metabolomic profiling of MAR vs health controls

OPLS-DA models showed a clear and distinctive clustering between MAR group and control group in both ESI+ and ESI- mode (P<0.05, Figure 1A and C). These models were then assessed by permutation analysis, and all permuted R2s were below or around 0.6 and all permuted Q2s were below 0, which means that all R2s and Q2s are lower than the original on the right (Figure 1B and D). Thus, this suggests these models fitting were valid and predictive. Finally, a total of 35 metabolites including 15 in the ESI+ mode and 20 in the ESI- mode responsible for distinguishing MAR patients from health controls were detected by UHPLC-MS analysis. Contribution plot ranks metabolites by their contribution to the model which is shown as a VIP. The top 10 metabolites with the highest VIP scores were identified as most potential discriminant metabolites and 6 related metabolic pathways were listed in the Table 2. According to metabolic pathway analyses, the most important pathways were arginine and proline metabolismand, glycerophospholipid metabolism, sphingolipid metabolism and fatty acid metabolism (Figure 4).

Metabolomic profiling of MSAR vs health controls

MSAR patients had different serum metabolic profiles in comparison with health controls by UHPLC-MS analysis in both ESI+ and ESI- mode (P<0.05, Figure 2A and C). The permutation analysis results showed that the models fitting were valid and predictive (Figure 2B and D). Compared with the control group, 59 metabolites including 29 in the ESI+ mode and 30 in the ESI- mode were expressed at significantly different levels in the MAR group. Results of top 10 potential discriminant metabolites and 9 related metabolic pathways were listed in the Table 3. The most important pathways including sphingolipid metabolism, pyrimidine metabolism and arginine and proline metabolism were revealed in the Figure 5.

Metabolomic profiling of MSAR vs MAR

In this study, AR patients were grouped into MSAR patients and MAR patients according to ARIA criteria, and the metabolic difference of these patients were further analyze. As shown in the Figure 3, serum metabolomics profiles of MSAR patients and MAR patients were significant different form each other in both ion mode (P<0.05, Figure 3A and C). The permutation analysis results exhibited good validation and predictability (Figure 3B and D). Compared with the MAR group, 30 metabolites including 17 in the ESI+ mode and 13 in the ESI- mode were detected at significantly different concentrations in the MSAR group. Results of top 10 potential discriminant metabolites and 8 related metabolic pathways were displayed in the Table 4. The most important pathways including fatty acids metabolism and sphingolipid metabolism were revealed in the Figure 6.

Metabolomic profiling and severity of AR

The distinctive metabolites among the three groups with good predictabilities (AUC>0.7) were included in Spearman's correlation analysis to evaluate their correlation with the severity of AR. As presented in the Table 5, sarcosine, sphingosine-1-phosphate (S1P), and cytidine levels were positively correlated with TNSS and VAS in the AR patients (P<0.05). However, linoleic acid levels were negatively correlated with TNSS and VAS (P<0.05).

Discussion

In the current prospective cohort study, we described a novel application of metabolomics in identifying the serum metabolic signatures and assessing the severity of AR. The OPLS-DA models showed that there were obvious discriminators between patients with different severity of AR and health controls. 35 and 59 metabolites responsible for differentiating MAR and MSAR patients from health controls, respectively, were identified. And 30 metabolites were found to be responsible for discriminating MSAR patients from MAR patients. After analyzing the relationships between the major discriminative metabolites with clinical parameters of AR patients, we observed that sarcosine, sphingosine-1-phosphate, cytidine and linoleic acid levels were associated with the severity of AR. These results showed that the identified potential serum biomarkers might be useful for diagnosing AR and developing objective indication for evaluating the severity of AR. We will next discuss the most significant metabolites and related metabolic pathways which may help us to better understand the underlying pathogenesis of AR and monitor the disease severity.

Most importantly, arginine and proline metabolism pathway was significantly perturbed among the most affected pathways in AR patients. Arginase and proline metabolism is of particular importance in the nitric oxide synthesis and integrally links to cellular respiration, metabolism, and inflammation [22-23]. A recent publication detected arginase and proline metabolism significant perturbations in the serum of commuters following traffic pollution exposures, and the researchers considered that arginase and proline metabolism dysfunction strongly associated with oxidative stress and inflammation in the air pollution toxicity [24]. Yang et al [25] found that the levels of arginine and its downstream products, such as ornithine, citrulline, creatine, creatinine, hydroxyproline and sarcosine were higher in the serum of asthma patients than in health controls, and they held that arginine and proline metabolism was the most important pathway in the development of asthma. Consistent with the previous report, we also observed that the levels of sarcosine and creatinine were higher in the serum of AR patients than in the health controls, and the levels of sarcosine correlated positively with TNSS and VAS. Arginine is an essential amino acid related to endothelial function, inflammation, and airway hyperresponsiveness, and higher levels of arginine and its downstream products can regulate T cell function and promote its activity, and act a critical role in several inflammatory diseases, including asthma and AR [22-23, 26]. Therefore, we speculated that arginine and proline metabolism might be involved in the development of AR and sarcosine could roughly be related to the severity of AR.

Our results also provide evidence for the sphingolipids metabolism alteration is involved in occurrence and progression of AR. Sphingolipids are ubiquitous components of the cell membrane and play an important role in cell growth, inflammation and tissue remodeling [27-28]. Among the numerous sphingolipids, S1P has received the greatest attention in allergic diseases and autoimmune diseases, as it has been implicated in the modulation of a variety of cell responses such as immune cell proliferation, differentiation and regulation [27, 29]. A previous study reported that S1P up-regulated the cytokine production, such as IL-12, IL-23 and IL-27, in activated murine bone marrow derived dendritic cells, and it might serve as a novel therapeutic target in the treatment of several inflammatory diseases [30]. In

another study, researchers found that the plasma levels of SIP were elevated in cystic fibrosis patients, and S1P levels correlated with routine laboratory parameters, lung function and clinical symptoms [31]. Kowal et al [29] analyzed targeted metabolites in the serum from 22 allergic asthma patients and 11 allergic rhinitis patients, and found that the sphingolipids metabolism was altered and the biosynthesis of S1P was augmented. In the present study, we observed that sphingolipids metabolism was disturbed and the S1P levels elevated in the AR patients, and the S1P levels were correlated positively with the disease severity of AR, which was in accordance with the results in the previous publications [29-30]. Our results support the hypothesis that alterations in the serum metabolites reflect the chronic activation of immune system in AR patients and that the disease severity is consistent with greater activation of the immune system. However, the mechanism underlying these manipulations has not been well clarified.

We firstly found that cytidine, identified form UHPLC-MS analysis, was associated with AR and it might be a novel marker and potential therapeutic target for AR. In our study, we observed that the levels of cytidine were elevated in the MAR and MSAR group, and the concentrations of cytidine were positively correlated with TNSS and VAS. Cytidine, a pyrimidine molecule, is considered as the precursor of the cytidine triphosphate (CTP), which is vital in the synthesis, interconversion and degradation of DNA, RNA and lipids [32-33]. Previous studies have found that abnormality of pyrimidine metabolism could influence the cell growth, development and differentiation, such as T cell and B cell [34]. A recent study demonstrated that interference of pyrimidine metabolism affected murine lymphocyte proliferation in vitro and attenuated the severity of experimental autoimmune arthritis [35]. Another study observed that the concentrations of 5,6-dihydorthymine were higher in the serum of current asthma patients compared with health controls, and the researchers believed that the alteration of pyrimidine metabolism might have relevance for asthma pathophysiology [36]. These events suggested that pyrimidine metabolism may play a role in the autoimmune diseases and allergic diseases. Therefore, we ultimately believed that cytidine was associated with AR, and that it might serve as a promising metablic biomarker for assessing the disease severity and evaluation of treatment.

Interestingly, we also found that fatty acids metabolism was dysregulated in all OPLS-DA models. In recent years, growing evidence suggests that fatty acids metabolism plays important roles in the modulation of immune responses in health and disease [37]. Most researchers hold that unsaturation fatty acids, especially polyunsaturated fatty acids exhibited potential protective effects on allergic inflammation, while saturation fatty acids promoted the inflammatory response [38]. In a recent animal experimental study, Lee et al [39] observed that oleic acid had anti-asthmatic effects such as the down-regulation of inflammatory cells and eosinophil in bronchial alveolar lavage fluid, IgE in serum. Several in vitro studies also demonstrated that unsaturation fatty acids could exert immunosuppressive effects on T cells, such as reducing its proliferation and activation in a dose-dependent manner [37, 40]. However, saturation fatty acids, such as palmitic acid, have been described as essential factors promoting T cell activation and cytokine secretion [41]. In addition, considerable evidence has shown that polyunsaturated fatty acids can modify mast cell functions and suppress its activation then reduce the production of cytokine or chemokine through receptors [42]. Therefore, we suppose that fatty acids metabolism may act an important role in the development of AR. In the current study, the concentrations of several

unsaturated fatty acids (linoleic acid, arachidic acid and trans-vaccenic acid) were lower in the serum of MAR or MSAR patients in comparison with health controls, while the concentrations of palmitic acid were elevated. Moreover, the levels of linoleic acid were correlated negatively with TNSS and VAS. Our results were in line with most previous studies. However, further studies should be conducted to confirm these results and to clarify the underlying mechanism of AR subtypes.

There are also several limitations in our study which may affect the reported findings. First, the total sample sizes were relatively small, and a validation cohort study was needed to confirm the conclusions. Second, all the recruited participants were from single-center with the same ethnicity and region, which might limit the applicability of our findings. Third, only one biological sample (serum) was used in the present study, future studies should collect other biological samples, such as urine and nasal lavage fluid, to further verify whether the identified differential metabolites were associated with AR. Last, we did not compare serum metabolites between MAR and MSAR patients, but it does not mean that there are no differential metabolites. Future studies with larger sample sizes using targeted approaches will be important to support and extend our present findings.

Conclusion

Our results suggest that serum metabolomics approaches can be successfully used to discriminate MSAR patients form MAR patients and health controls and establish a metabolite signature associated with the severity of AR. These results will be useful for diagnosing AR and developing objective indication for evaluating the severity of AR.

Abbreviations

AR: Allergic rhinitis; MAR: Mild allergic rhinitis; MSAR: Moderate to severe allergic rhinitis; UHPLC-MS: Ultra-high performance liquid chromatography-mass spectrometry; OPLS-DA: Orthogonal partial least square-discriminate analysis; S1P: Sphingosine-1-phosphate; TNSS: Total nasal symptom score; VAS: Visual analogue scale; ARIA: Allergic rhinitis and its impact on asthma; IgE: Immunoglobulin E; HDM: House dust mite; BMI: Body mass index; ESI: Electrospray ionization; VIP: Variable importance for project; AUC: Area Under the Curve.

Declarations

Acknowledgments

The authors would like to thank the patients, investigators and staff at participating centers in this study.

Authors' contributions

All authors contributed equally to the design of the study, data analysis and drafting, and revising the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by National Natural Science Foundation of China (No. 81770985, No. 81770985 and No. 81800917) and Natural Science Foundation of Hunan Province (No.2020JJ4910 and No. 2018JJ2662).

Availability of data and materials

The datasets generated and/or analyzed during the current study are not available for public disclosure.

Ethics approval and consent to participate

All participants gave informed consent and the study was approved by the ethical committee of Xiangya Hospital of Central South University (protocol no. 2019050725).

Consent for publication

Not applicable.

Competing interests

The authors declare no financial or other conflicts of interest.

References

1. Ai J, Xie Z, Qing X, Li W, Liu H, Wang T, et al. Clinical Effect of Endoscopic Vidian Neurectomy on Bronchial Asthma Outcomes in Patients with Coexisting Refractory Allergic Rhinitis and Asthma. Am J Rhinol Allergy. 2018; 32:139-46.
2. Chen X, Xie ZH, Lv YX, Tang QP, Zhang H, Zhang JY, et al. A proteomics analysis reveals that A2M might be regulated by STAT3 in persistent allergic rhinitis. Clin Exp Allergy. 2015;46:813-24.
3. Meng Y, Wang C, and Zhang L. Recent developments and highlights in allergic rhinitis. Allergy. 2019; 74:2320-8.
4. Shen Z, Tan G, Zhong Z, Ding S, and Wang F. Interactive Network Platform Improves Compliance and Efficacy of Subcutaneous Immunotherapy for Patients with Allergic Rhinitis. Patient Prefer Adherence. 2019; 13:2101-10.
5. Zhang Y, and Zhang L. Increasing Prevalence of Allergic Rhinitis in China. Allergy Asthma Immunol Res. 2019; 11:156-69.
6. Brozek JL, Bousquet J, Agache I, Agarwal A, Bachert C, Bosnic-Anticevich S, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines-2016 revision. J Allergy Clin Immunol. 2017; 140:950-8.
7. Del Cuvillo A, Santos V, Montoro J, Bartra J, Davila I, Ferrer M, et al. Allergic rhinitis severity can be assessed using a visual analogue scale in mild, moderate and severe. Rhinology. 2017; 55:34-8.

8. Hou J, Lou H, Wang Y, He F, Cao F, Wang C, et al. Nasal ventilation is an important factor in evaluating the diagnostic value of nasal nitric oxide in allergic rhinitis. International forum of allergy & rhinology. 2018; 8:686-94.
9. Han MW, Kim SH, Oh I, Kim YH, and Lee J. Serum IL-1beta can be a biomarker in children with severe persistent allergic rhinitis. Allergy, asthma, and clinical immunology : official journal of the Canadian Society of Allergy and Clinical Immunology. 2019; 15:58.
10. Pirayesh A, Shahsavan S, Zargari Samani O, Shirzad H, Amani S, Zamani MA, et al. Differential Expression of Fas in Moderate/Severe and Mild Persistent Allergic Rhinitis and Its Correlation With Pathological Parameters. Am J Rhinol Allergy. 2019; 33:286-93.
11. Chen C, Luo F, Wu P, Huang Y, Das A, Chen S, et al. Metabolomics reveals metabolite changes of patients with pulmonary arterial hypertension in China. Journal of cellular and molecular medicine. 2020; 24:2484-96.
12. Zhan X, Long Y, and Lu M. Exploration of variations in proteome and metabolome for predictive diagnostics and personalized treatment algorithms: Innovative approach and examples for potential clinical application. Journal of proteomics. 2018; 188:30-40.
13. Adamko DJ, Nair P, Mayers I, Tsuyuki RT, Regush S, and Rowe BH. Metabolomic profiling of asthma and chronic obstructive pulmonary disease: A pilot study differentiating diseases. J Allergy Clin Immunol. 2015; 136:571-80.e3.
14. Ning P, Zheng Y, Luo Q, Liu X, Kang Y, Zhang Y, et al. Metabolic profiles in community-acquired pneumonia: developing assessment tools for disease severity. Critical care (London, England). 2018; 22:130.
15. Spertini F. Metabolomics and allergy: Opening Pandora's box. J Allergy Clin Immunol. 2020; 145:782-4.
16. Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. Nat Protoc. 2011; 6:1060-83.
17. Liu S, Liang Y-Z, and Liu H-T. Chemometrics applied to quality control and metabolomics for traditional Chinese medicines. J Chromatogr B Analyt Technol Biomed Life Sci. 2016; 1015-1016:82-91.
18. Zhao H, Cheng N, Wang Q, Zhou W, Liu C, Liu X, et al. Effects of honey-extracted polyphenols on serum antioxidant capacity and metabolic phenotype in rats. Food Funct. 2019; 10:2347-58.
19. Kuhl C, Tautenhahn R, Böttcher C, Larson TR, and Neumann S. CAMERA: an integrated strategy for compound spectra extraction and annotation of liquid chromatography/mass spectrometry data sets. Anal Chem. 2012; 84:283-9.
20. Yang Y, Wu Z, Li S, Yang M, Xiao X, Lian C, et al. Targeted Blood Metabolomic Study on Retinopathy of Prematurity. Invest Ophthalmol Vis Sci. 2020; 61:12.
21. Wang W, Zhao L, He Z, Wu N, Li Q, Qiu X, et al. Metabolomics-based evidence of the hypoglycemic effect of Ge-Gen-Jiao-Tai-Wan in type 2 diabetic rats via UHPLC-QTOF/MS analysis. J

Ethnopharmacol. 2018; 219:299-318.
22. King NE, Rothenberg ME, and Zimmermann N. Arginine in asthma and lung inflammation. J Nutr. 2004; 134.
23. Scott JA, and Grasemann H. Arginine metabolism in asthma. Immunol Allergy Clin North Am. 2014; 34:767-75.
24. Liang D, Ladva CN, Golan R, Yu T, Walker DI, Sarnat SE, et al. Perturbations of the arginine metabolome following exposures to traffic-related air pollution in a panel of commuters with and without asthma. Environ Int. 2019; 127:503-13.
25. Quan-Jun Y, Jian-Ping Z, Jian-Hua Z, Yong-Long H, Bo X, Jing-Xian Z, et al. Distinct Metabolic Profile of Inhaled Budesonide and Salbutamol in Asthmatic Children during Acute Exacerbation. Basic Clin Pharmacol Toxicol. 2017; 120:303-11.
26. Xu W, Comhair SAA, Janocha AJ, Lara A, Mavrakis LA, Bennett CD, et al. Arginine metabolic endotypes related to asthma severity. PLoS ONE. 2017; 12:e0183066.
27. Mohammed S, and Harikumar KB. Sphingosine 1-Phosphate: A Novel Target for Lung Disorders. Front Immunol. 2017; 8:296.
28. Rivera J, Proia RL, and Olivera A. The alliance of sphingosine-1-phosphate and its receptors in immunity. Nat Rev Immunol. 2008; 8:753-63.
29. Kowal K, Żebrowska E, and Chabowski A. Altered Sphingolipid Metabolism Is Associated With Asthma Phenotype in House Dust Mite-Allergic Patients. Allergy, asthma & immunology research. 2019; 11:330-42.
30. Schaper K, Kietzmann M, and Bäumer W. Sphingosine-1-phosphate differently regulates the cytokine production of IL-12, IL-23 and IL-27 in activated murine bone marrow derived dendritic cells. Mol Immunol. 2014; 59:10-8.
31. Halilbasic E, Fuerst E, Heiden D, Japtok L, Diesner SC, Trauner M, et al. Plasma Levels of the Bioactive Sphingolipid Metabolite S1P in Adult Cystic Fibrosis Patients: Potential Target for Immunonutrition? Nutrients. 2020; 12.
32. Chen HW, Zhou W, Liao Y, Hu SC, Chen TL, and Song ZC. Analysis of metabolic profiles of generalized aggressive periodontitis. J Periodont Res. 2018; 53:894-901.
33. Zhu X-R, Yang F-Y, Lu J, Zhang H-R, Sun R, Zhou J-B, et al. Plasma metabolomic profiling of proliferative diabetic retinopathy. Nutr Metab (Lond). 2019; 16:37.
34. Garavito MF, Narváez-Ortiz HY, and Zimmermann BH. Pyrimidine Metabolism: Dynamic and Versatile Pathways in Pathogens and Cellular Development. J Genet Genomics. 2015; 42:195-205.
35. Peres RS, Santos GB, Cecilio NT, Jabor VAP, Niehues M, Torres BGS, et al. Lapachol, a compound targeting pyrimidine metabolism, ameliorates experimental autoimmune arthritis. Arthritis Res Ther. 2017; 19:47.
36. Kelly RS, Sordillo JE, Lasky-Su J, Dahlin A, Perng W, Rifas-Shiman SL, et al. Plasma metabolite profiles in children with current asthma. Clin Exp Allergy. 2018; 48:1297-304.

37. Wang X, and Kulka M. n-3 Polyunsaturated fatty acids and mast cell activation. J Leukoc Biol. 2015; 97:859-71.
38. Venter C, Meyer RW, Nwaru BI, Roduit C, Untersmayr E, Adel-Patient K, et al. EAACI position paper: Influence of dietary fatty acids on asthma, food allergy, and atopic dermatitis. Allergy. 2019; 74:1429-44.
39. Lee S-Y, Bae C-S, Seo N-S, Na C-S, Yoo HY, Oh D-S, et al. Camellia japonica oil suppressed asthma occurrence via GATA-3 & IL-4 pathway and its effective and major component is oleic acid. Phytomedicine. 2019; 57:84-94.
40. Arita M. Eosinophil polyunsaturated fatty acid metabolism and its potential control of inflammation and allergy. Allergol Int. 2016; 65 Suppl:S2-S5.
41. Radzikowska U, Rinaldi AO, Çelebi Sözener Z, Karaguzel D, Wojcik M, Cypryk K, et al. The Influence of Dietary Fatty Acids on Immune Responses. Nutrients. 2019; 11.
42. Yu G, and Björkstén B. Polyunsaturated fatty acids in school children in relation to allergy and serum IgE levels. Pediatr Allergy Immunol. 1998; 9:133-8.

Tables

Table 1 Clinical characteristics of participants

Variable	Control (n=29)	MAR (n=30)	MSAR (n=42)	*P* value
Gender, male/female	14/15	17/13	22/20	0.914
Age, years	28.5±8.5	28.2±9.6	30.4±8.4	0.518
BMI, kg/m^2	22.2±1.8	22.8±1.8	22.1±1.6	0.224
Disease duration, years	NA	5.5±2.8	4.9±2.2	0.300
Total IgE, IU/mL	79.2±29.1	212.4±83.6	430.4±155.7	<0.001
Special IgE for HDM, IU/mL	0.2±0.1	0.7±0.2	5.8±3.9	<0.001
Blood eosinophil counts, cells/uL	81.2±24.0	176.1±97.8	451.2±192.4	<0.001
TNSS	1.2±0.7	3.9±1.0	9.5±1.7	<0.001
VAS	1.3±0.6	3.1±0.9	6.9±1.5	<0.001

MAR mild allergic rhinitis, *MSAR* moderate-severe allergic rhinitis, *BMI* body mass index,

HDM house dust mite, *TNSS* total nasal symptom score, *NA* not applicable.

Table 2 Top ten metabolites with different variations discriminating MAR patients from health controls

Metabolites	Ion mode	VIP	P-value	Regulation	AUC	Pathway
Sarcosine	Positive	3.24	0.005	up	0.77	Arginine and proline metabolism
Palmitic acid	Positive	2.74	0.000	up	0.87	Fatty acid metabolism
5'-Methylthioadenosine	Positive	2.58	0.002	up	0.60	Cysteine and methionine metabolism
Triethanolamine	Negative	2.16	0.047	up	0.79	Glycerophospholipid metabolism
5-Methoxyindoleacetate	Negative	1.86	0.004	up	0.75	Tryptophan metabolism
Trans-Vaccenic acid	Positive	1.79	0.004	down	0.93	Fatty acid metabolism
Creatinine	Negative	1.73	0.000	up	0.78	Arginine and proline metabolism
S1P	Negative	1.70	0.019	up	0.91	Sphingolipid metabolism
Arachidic acid	Positive	1.62	0.014	down	0.54	Fatty acids metabolism
L-Methionine	Negative	1.61	0.022	down	0.49	Cysteine and methionine metabolism

MAR mild allergic rhinitis, *VIP* variable importance for project, *AUC* area under the curve, *S1P* sphingosine-1-phosphate.

Table 3 Top ten metabolites with different variations discriminating MSAR patients from health controls

Metabolites	Ion mode	VIP	P-value	Regulation	AUC	Pathway
S1P	Positive	1.97	0.005	up	0.89	Sphingolipid metabolism
2-Oxoadipic acid	Negative	2.36	0.002	up	0.68	Tryptophan metabolism
Phosphorylcholine	Negative	2.19	0.002	up	0.86	Glycerophospholipid metabolism
Cytidine	Negative	2.01	0.000	up	0.83	Pyrimidine metabolism
Betaine	Positive	1.97	0.000	up	0.84	Glycine, serine and threonine metabolism
Sarcosine	Negative	1.95	0.002	up	0.92	Arginine and proline metabolism
1,3-Diaminopropane	Positive	1.85	0.007	up	0.61	beta-Alanine metabolism
Taurocholic acid	Positive	1.82	0.006	down	0.74	Taurine and hypotaurine metabolism
Linoleic acid	Negative	1.78	0.026	down	0.79	Fatty acid metabolism
Cis-9-Palmitoleic acid	Positive	1.75	0.005	up	0.64	Fatty acid metabolism

MSAR moderate-severe allergic rhinitis, *VIP* variable importance for project, *AUC* area under the curve, *S1P* sphingosine-1-phosphate

Table 4 Top ten metabolites with different variations discriminating MSAR from MAR

Metabolites	Ion mode	VIP	P-value	Regulation	AUC	Pathway
Linoleic acid	Positive	2.79	0.049	down	0.77	Fatty acids metabolism
Betaine	Positive	2.24	0.034	up	0.60	Glycine, serine and threonine metabolism
Coumarin	Positive	2.11	0.012	down	0.63	Phenylpropanoid biosynthesis
S1P	Negative	2.06	0.009	up	0.72	Sphingolipid metabolism
Palmitoleic acid	Positive	1.97	0.016	down	0.75	Fatty acids metabolism
Trans-Vaccenic acid	Positive	1.89	0.003	down	0.65	Fatty acids metabolism
D-Glucurono-6,3-lactone	Negative	1.88	0.000	up	0.59	Ascorbate and aldarate metabolism
Sarcosine	Negative	1.83	0.007	up	0.91	Arginine and proline metabolism
Cytidine	Positive	1.79	0.017	up	0.74	Pyrimidine metabolism
Pyroglutamic acid	Negative	1.72	0.014	down	0.81	Glutathione metabolism

MAR mild allergic rhinitis, *MSAR* moderate-severe allergic rhinitis, *VIP* variable importance for project, *AUC* area under the curve, *S1P* sphingosine-1-phosphate

Table 5 Correlation of serum metabolites with severity of AR

Metabolites	TNSS		VAS	
	r	*P* value	r	*P* value
Sarcosine	0.551	0.012	0.376	0.040
Palmitic acid	0.489	0.137	0.413	0.107
Triethanolamine	-0.212	0.170	0.301	0.019
Betaine	0.431	0.049	0.298	0.202
5-Methoxyindoleacetate	-0.204	0.765	0.376	0.046
Trans-Vaccenic acid	-0.312	0.031	-0.178	0.099
Creatinine	0.702	0.129	0.561	0.049
S1P	0.821	0.004	0.673	0.030
Phosphorylcholine	0.378	0.418	0.277	0.031
Cytidine	0.598	0.028	0.312	0.017
Diethanolamine	0.242	0.782	0.134	0.458
Cis-9,10-Epoxystearic acid	0.366	0.232	-0.221	0.022
Taurocholic acid	-0.207	0.651	-0.319	0.562
Linoleic acid	-0.792	0.031	-0.493	0.041
Palmitoleic acid	-0.377	0.052	-0.274	0.093
Pyroglutamic acid	-0.134	0.202	-0.307	0.089

AR allergic rhinitis, *TNSS* total nasal symptom score, *VAS* visual analogue scale, *HDM* house dust mite, *S1P* sphingosine-1-phosphate

Figures

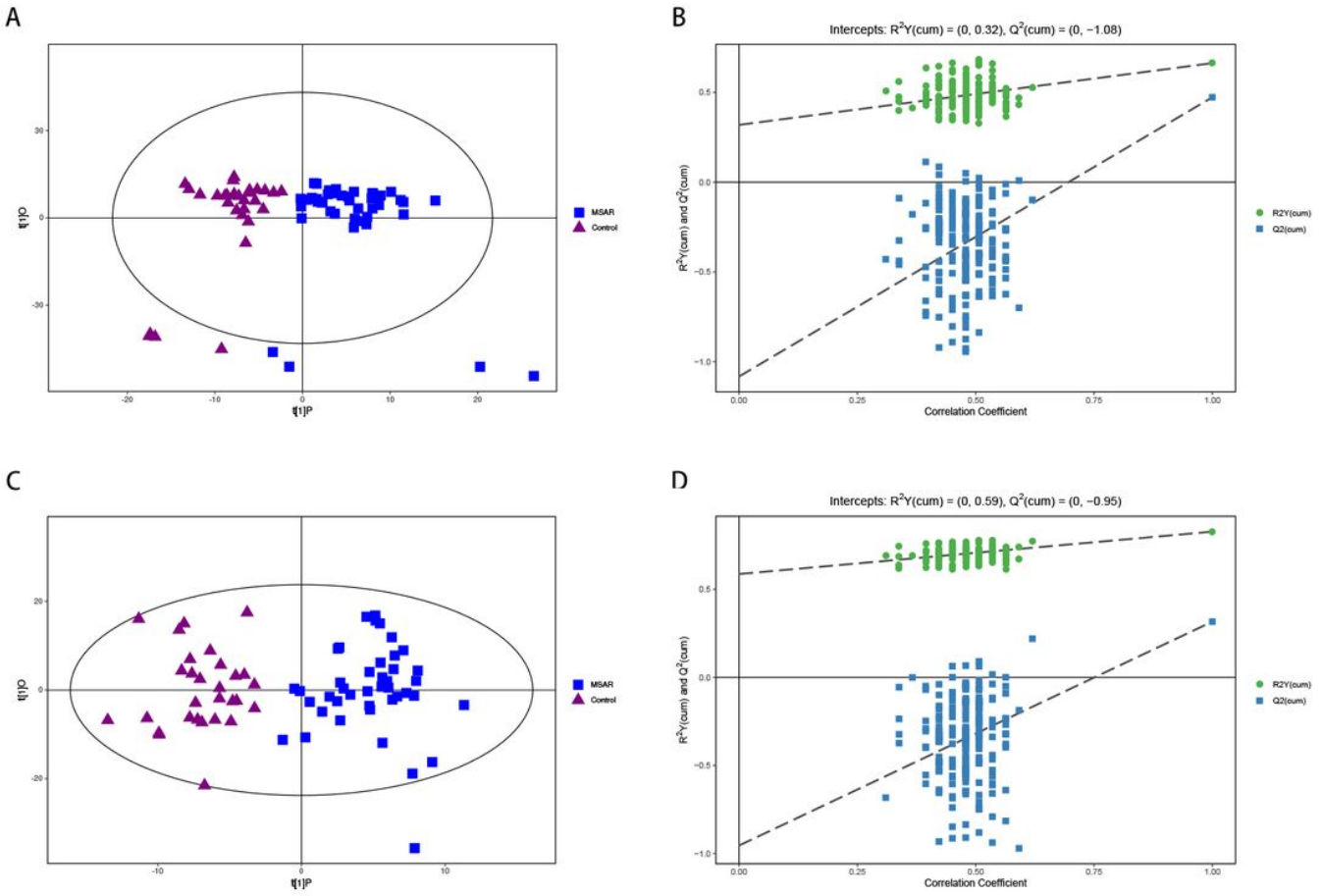

Figure 1

OPLS-DA-MSAR vs control

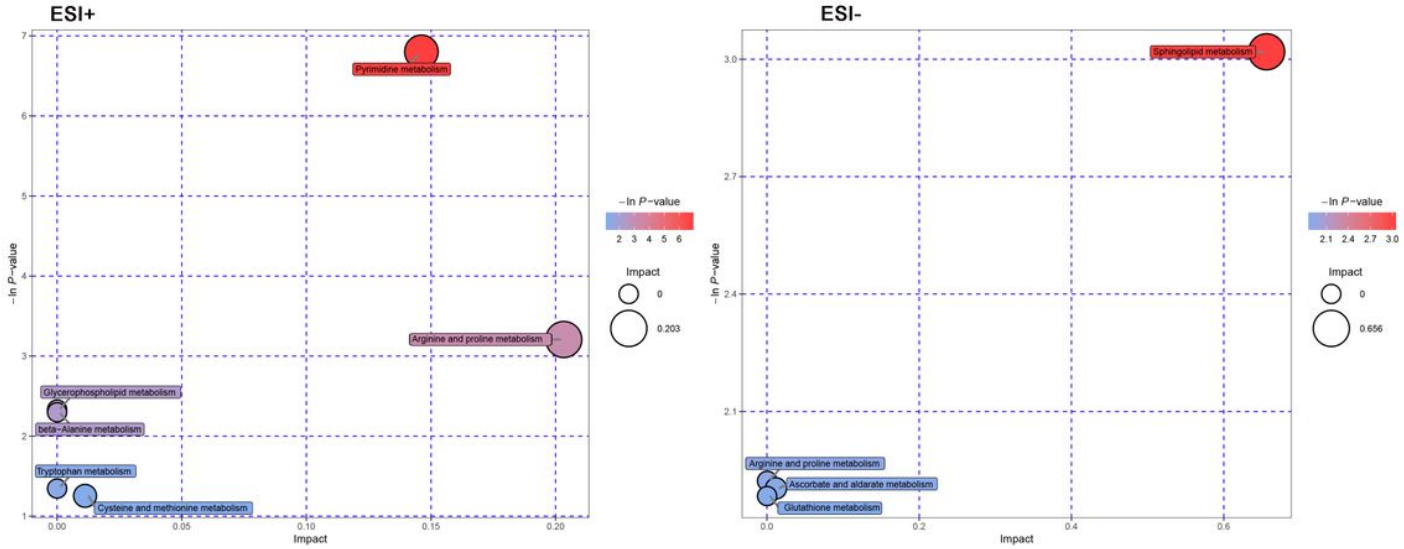

Figure 2

MSAR vs MAR

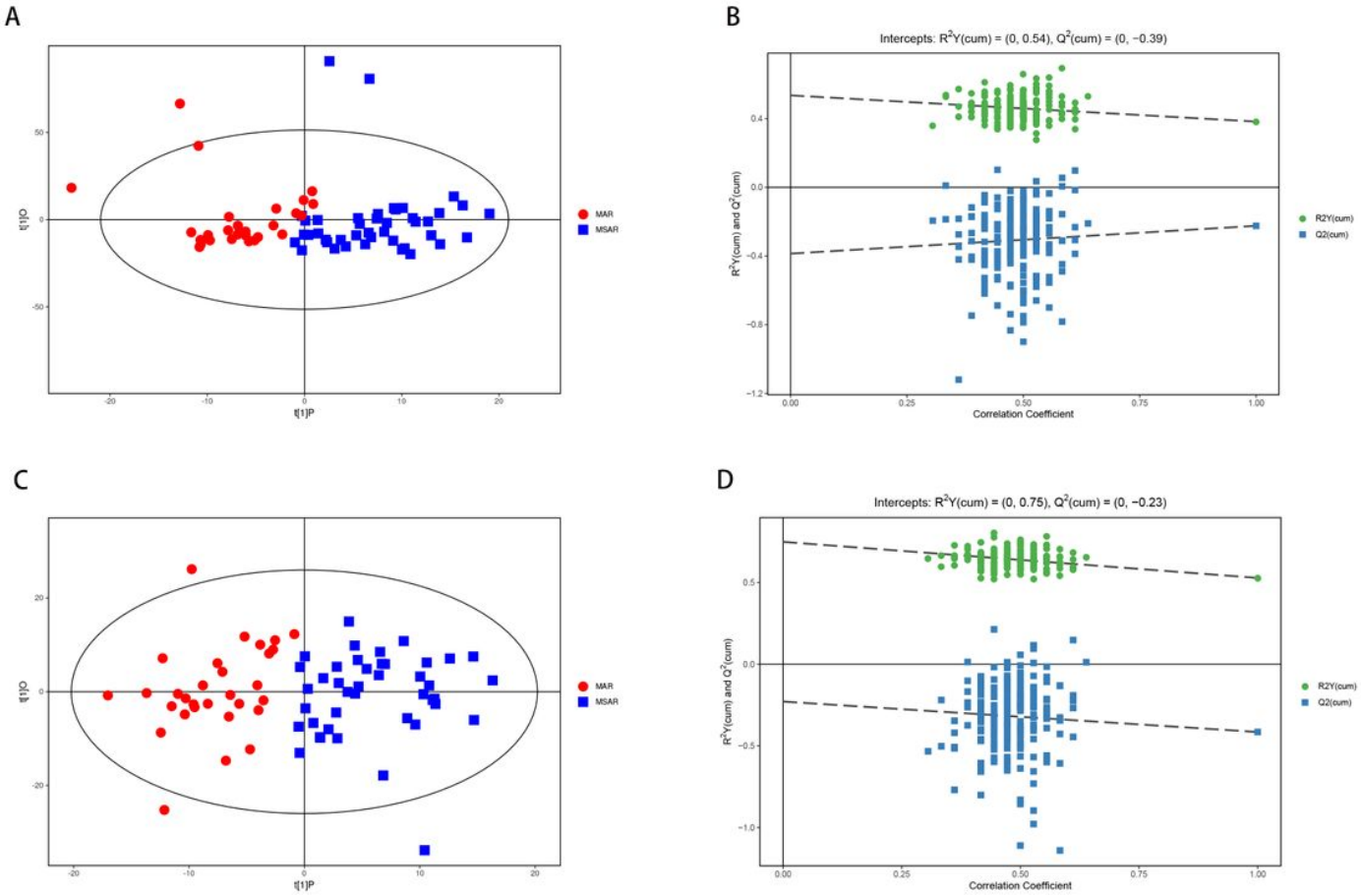

Figure 3

OPLS-DA-MAR vs MSAR

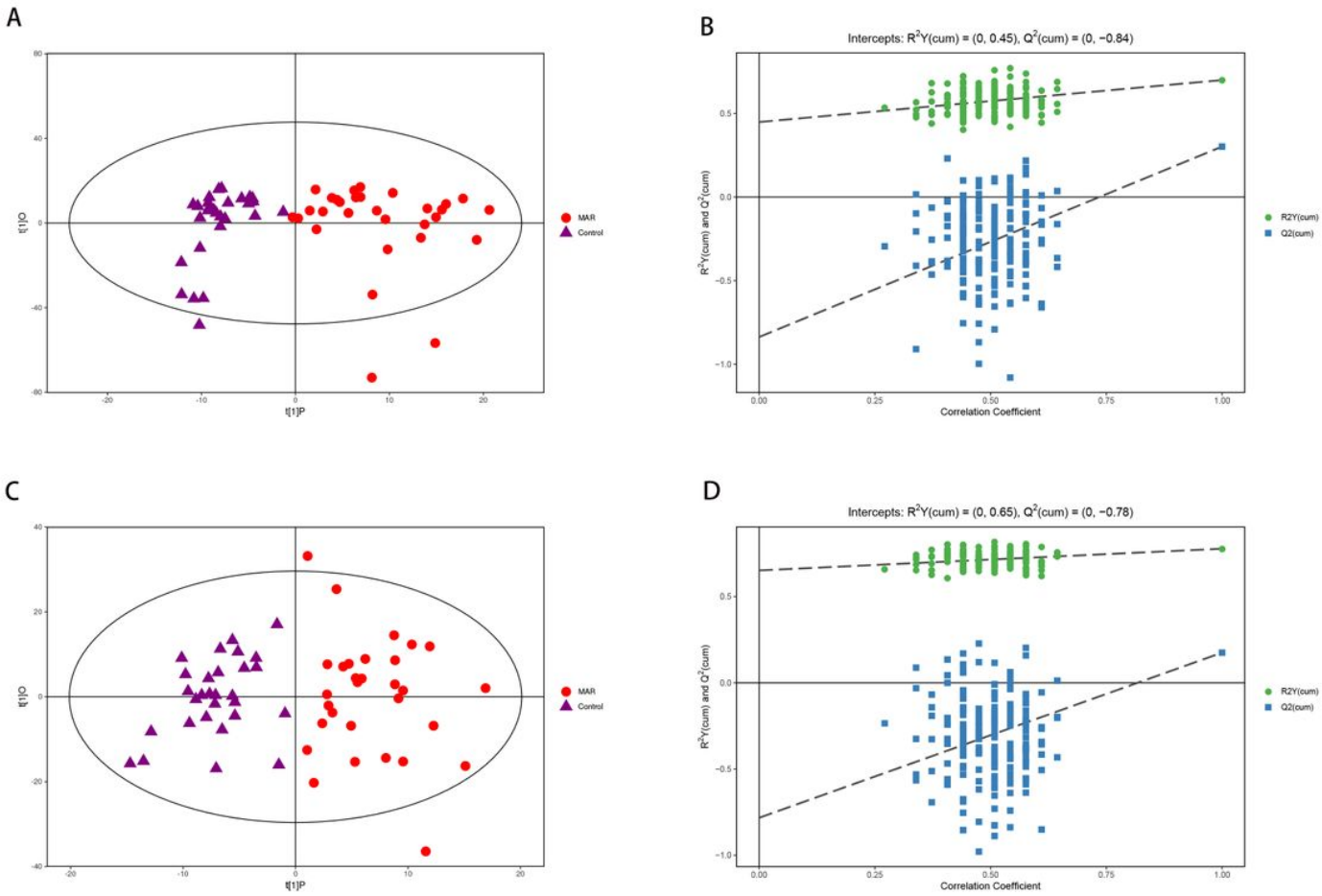

Figure 4

OPLS-DA-MAR vs control

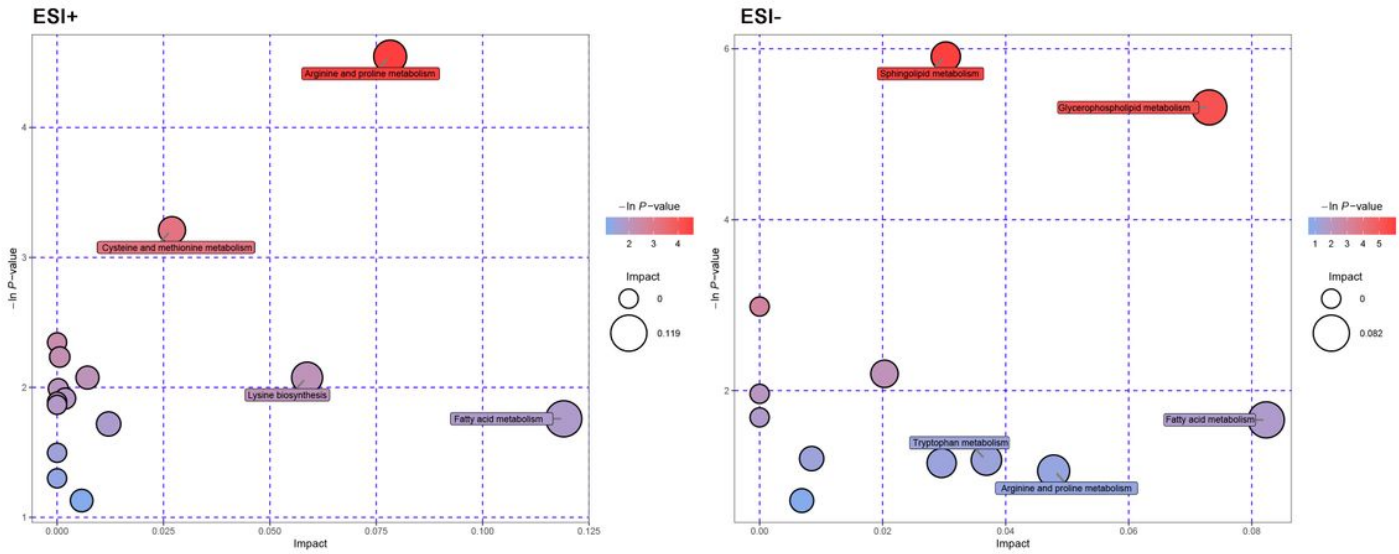

Figure 5

MAR vs control

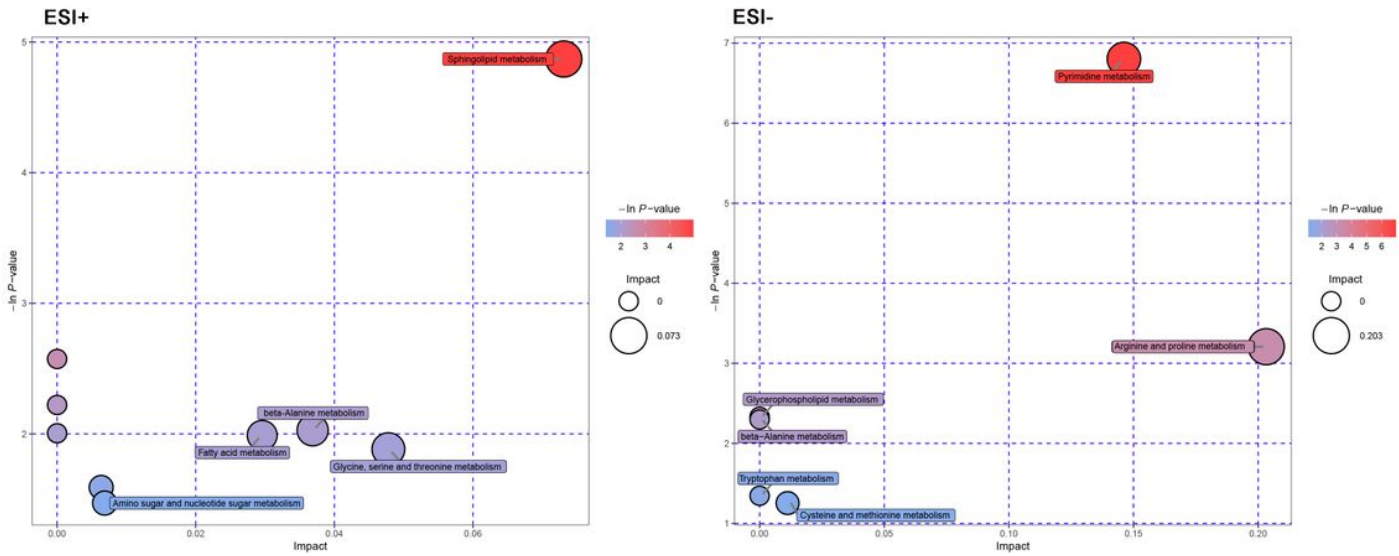

Figure 6

MSAR vs control