



Prediction of sublingual immunotherapy efficacy in allergic rhinitis by serum metabolomics analysis

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ABSTRACT

Background: Allergen-specific immunotherapy (ASIT) is currently the only therapy for allergic rhinitis (AR) that can induce immune tolerance to allergens. However, the course of ASIT is long and there is no objective biomarker to predict treatment efficacy. The present study aimed to explore potential biomarkers predictive of efficacy of AIT based on serum metabolomics profiles.

Methods: This prospective study recruited 72 consecutive eligible patients who were assigned to receive sublingual immunotherapy (SLIT). Serum samples were collected prior to SLIT and utilized to obtain metabolomics profiling by applying ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS). Treatment response was determined 3 years after SLIT, and patients were divided into effective group and ineffective group. Orthogonal partial least square-discriminate analysis (OPLS-DA) was performed to evaluate the metabolite differences between two groups.

Results: Sixty-eight patients completed the whole SLIT, 39 patients were categorized into effective group and 29 patients were classified into ineffective group. A total of 539 metabolites were obtained, and 197 of which were identified as known substances. Using these 197 known metabolites, the OPLS-DA results showed that effective group and ineffective group exhibited distinctive metabolite signatures and metabolic pathways. Six metabolites including lactic acid, ornithine, linolenic acid, creatinine, arachidonic acid and sphingosine were identified to exhibit good performance in predicting the efficacy of SLIT, and these metabolite changes mainly involved glycolysis and pyruvate metabolism, arginine and proline metabolism and fatty acid metabolism pathways.

Conclusion: By metabolomics analysis, we identified several serum biomarkers that can reliably and accurately predict the efficacy of SLIT in AR patients. The discriminative metabolites and related metabolic pathways contributed to better understand the mechanisms of SLIT in AR patients.

1. Introduction

Allergic rhinitis (AR) is a type I allergic disease caused by common allergens such as dust mite, pollen and animal hair [1]. Epidemiological studies have shown that AR was prevalent in a billion people worldwide, and the prevalence still continued to increase [1,2]. The common treatments for AR include allergen avoidance, patient education,

pharmacotherapy and allergen-specific immunotherapy (ASIT) [3]. Although allergen avoidance and patient education are the basic management for most AR patients, they can't relief allergic symptoms [2]. Pharmacotherapy has been demonstrated to be effective to control allergic symptoms, and the first-line used drugs for AR includes nasal/oral antihistamines, glucocorticoids and antihistamines [1]. However, pharmacological interventions can't alter the natural course of AR, and

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patients need continuation of drugs consumption for lifetime to control the allergic symptoms [4]. In addition, a large proportion of patients fail to adequately respond to pharmacological treatments, and long-term pharmacotherapy will increase the risk of adverse reactions, including local adverse events (xeromycteria, hemorrhinia and nasal septal perforation, etc) and systemic events (somnolence, arrhythmia and hypercortical syndrome, etc) [1,4,5]. ASIT is the only disease-modifying and curative option for AR, and it can be conducted by subcutaneous (SCIT) or sublingual (SLIT), but more and more AR patients prefer to choosing SLIT owing to its equivalent efficacy, convenience, and reduced pain and side effects [6,7]. In spite of its safety and efficacy, SLIT generally takes 3 years, and not all AR patients respond to this therapy, the effective rate always fluctuates from 50.0% to 80.0% with varying definitions of efficacy [8]. Treating AR patients who do not respond to SLIT is unavailing and will increase resources waste. Therefore, it is extremely important to predict the therapeutic efficacy and identify non-responder patients before administrating SLIT.

Metabolomics, a branch of omics technology, systematically analyzes the concentrations of all low-molecular mass metabolites in the living systems at cellular level [9]. Metabolites are the end products and relatively stable and can be quantified, which provides opportunities to establish a powerful exploratory tool for monitoring disease status, and help to explain disease pathophysiological mechanisms [10]. Metabolomics signatures from serum or urine samples have been widely used in many diseases to identify markers for early disease detection and therapeutic efficacy prediction [11,12]. Recently, several studies have applied metabolomics analyses to explore the metabolic changes in inflammatory and allergic diseases, and successfully identified some potential metabolites and key metabolic pathways which might improve the understanding of these disorders and develop novel therapy target [13,14]. However, no previous study has employed metabolomics to investigate the mechanisms of SLIT and predict its efficacy in AR patients.

Therefore, the aim of this study was to explore several serum metabolites to accurately predict the response of SLIT in AR patients by applying ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS).

2. Methods

2.1. Study design and patient population

This single-center prospective study was conducted to explore the serum metabolic biomarkers to predict the efficacy of SLIT. All patients recruited in the present study were assigned to receive SLIT between January 2017 and April 2017, and the serum samples were prospectively collected before treatment. The diagnosis of AR was conducted by allergy specialists according to the allergic rhinitis and its impact on asthma (ARIA) guidelines [15]. All AR patients met following inclusion criteria: moderated or severe persistent AR; duration of disease >2 years; a positive skin test to *Dermatophagoides farina* (*Der f*) and/or *Dermatophagoides pteronyssinus* (*Der p*) (at least ++). All patients underwent a brief interview to exclude those with other allergic diseases, systemic or nasal steroid treatment, inflammatory or septic diseases, autoimmune diseases, age < 18 years, pregnant condition, immunotherapy, and use of anti-allergic drugs during the 4 weeks that preceded the study. Demographic and clinical information were collected, including sex, age, body mass index (BMI), disease duration and serum total IgE, baseline total nasal symptom score (TNSS), visual analogue scale (VAS), medication score (MS); symptom and medication score (SMS).

2.2. Immunotherapy

Eligible patients were self-administered with standardized allergen *Der f* drops at home which were provided by Wolwopharma

Biotechnology Company (Zhejiang, China). According to the manufacturer's instruction, the biologically standardized extracts were labeled with the concentration of total protein (labeled from No.1 to No.5 containing proteins of 1, 10, 100, 333, and 1000 µg/mL, respectively). Patients were instructed to keep the drops under tongue for 2–3 min then spat it out. The first dose was given in the outpatient under the guide of allergy specialists, patients should be observed for >30 min before they leave the hospital. The step-up dosage and maintenance protocol was described as previous study [8]. When SLIT cannot completely control the allergic symptoms, particularly when the allergen load is heavy, rescue medication will be prescribed.

2.3. Patient education, follow-up and clinical efficacy assessment

In order to improve patient compliance, initial patient education was conducted at the first two months in the first year of SLIT as previous suggested [8], including giving patients information on the disease characteristics of AR, the course, costs, efficacy and safety of SLIT by publicity brochures. Follow-up education was performed through follow-up visits or telephone, and allergy specialists collected information on patient's recent clinical symptoms and drug consumption, adjusted the SLIT program based on efficacy assessment, guided patients how to avoid and manage adverse reactions. In addition, all patients were provided with medical consulting services through telephone, short message and WeChat groups. All patients were followed up for at least 3 years and asked to record the symptom and drug consumption during the whole treatment. The clinical effectiveness of SLIT was assessed on the basis of improvement of clinical symptoms and the reduction in the consumption of combined drugs after 3 years of treatment as previous study described [16,17]. MS was defined as the sum of medication consumption in the previous week, and was recorded according to the World Allergy Organization recommendations [18]: 1 point for oral or intranasal antihistamines; 2 points for nasal glucocorticoids; and 3 points for oral glucocorticoids. The final MS was calculated based on the arithmetic mean score of each day. SMS was described as the sum of TNSS and final MS. When patient obtained at least 30% reduction of SMS compared to baseline was defined as effective SLIT, otherwise the SLIT was regarded as ineffective [17,19].

2.4. Serum sample collection and preparation

Venous blood samples were prepared as previous publications described [20]. Fasting peripheral blood samples from AR patients and healthy volunteers were collected with vacuum blood collection tubes without anticoagulation or coagulant in the morning before onset of SLIT, and the tubes were stored for 1 h at room temperature. All blood samples were centrifuged at 3000 rpm for 10 min at 4 °C, serum were collected and stored at −80 °C in equal aliquots for subsequent analysis. The serum samples were prepared for UHPLC-MS analysis by mixing 100 µL of serum sample with 300 µL 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 °C for 1 h and centrifuged at 12,000 rpm for 15 min at 4 °C. 100 µL of supernatant was transferred to a fresh glass vial for UHPLC-MS analysis [21,22]. 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 previous study described [23].

2.5. UHPLC-MS analysis

The UHPLC separation was performed using a 1290 Infinity series UHPLC System (Waters Corporation, Milford, MA, USA), equipped with a UPLC BEH Amide column (2.1 mm × 100 mm, 1.7 µm). 25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water were applied as phase A and B. The analysis was performed with elution

Table 1
Base characteristics of AR patients treated with SLIT.

Variables	Effective group (n = 39)	Ineffective group (n = 29)	P value
Sex			0.464
Male	24 (61.5%)	15 (51.7%)	
Female	15 (38.5%)	14 (48.3%)	
Age, years	30.3 ± 9.3	32.7 ± 8.3	0.266
BMI, kg/m ²	22.4 (19.9–23.7)	22.1 (19.7–23.9)	0.512
Disease duration, years	5.0 (3.0–7.0)	5.0 (3.5–7.0)	0.788
Serum total IgE, IU/ml	381.6 (309.3–482.6)	356.8 (320.8–478.5)	0.796
Baseline TNSS	10 (9–11)	10 (9–11)	0.508
Baseline VAS	8 (6–9)	8 (6–9)	0.952
Baseline MS	1.6 (1.1–1.9)	1.4 (1.1–1.7)	0.380
Baseline SMS	11.7 (10.6–12.3)	11.6 (10.9–12.3)	0.724

SLIT, sublingual immunotherapy; BMI, body mass index; SD, standard deviation; TNSS, total nasal symptom score; VAS, visual analogue scale; MS, medication score; SMS, symptom and medication score.

gradient as previously described [24]. The Triple TOF 6600 mass spectrometry (AB Sciex, Boston, MA, USA) was applied for its ability to obtain MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. 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 °C, declustering potential as 60 V, ion spray voltage floating (ISVF) as 5000 V.

2.6. Data processing and analysis

MS raw data (.wiff) files were converted to the mzXML format by Proteo Wizard, and analyzed by R package version 3.2. The process includes peak extraction, alignment and integration. Minfrac and cutoff are set as 0.5 and 0.3 respectively as previous study described [24,25]. Metabolites identification referred to In-house MS2 database. 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 conducted to explore potential biomarkers that contributed to the metabolic differences between effective and ineffective groups [26]. The quality of models was confirmed by R2Ycum and Q2cum to assess the goodness of fit and prediction, respectively. In addition, 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 calculated according to the variable importance for project (VIP) values (VIP > 1.0) and P-values (P < 0.05) [27,28], and we applied threshold of 2 for VIP to explore metabolites that contributed more to the efficacy prediction. Receiver operating characteristics (ROC) analysis was performed to evaluate the performance of the identified combination, and area under the curve (AUC) was calculated to assess the sensitivity and specificity. The metabolic pathway was analyzed using MetaboAnalyst 3.0.

2.7. Statistical analysis

Continuous variables with normal distribution are presented as mean ± standard deviation (SD), median and 1st to 3rd quartile for variables with non-normal distribution, and number (percentage) for discontinuous variables. Student's *t* test or Mann-Whitney *U* test was performed for testing differences between continuous variables, and Chi-square test was used for categorical variables. All above statistical

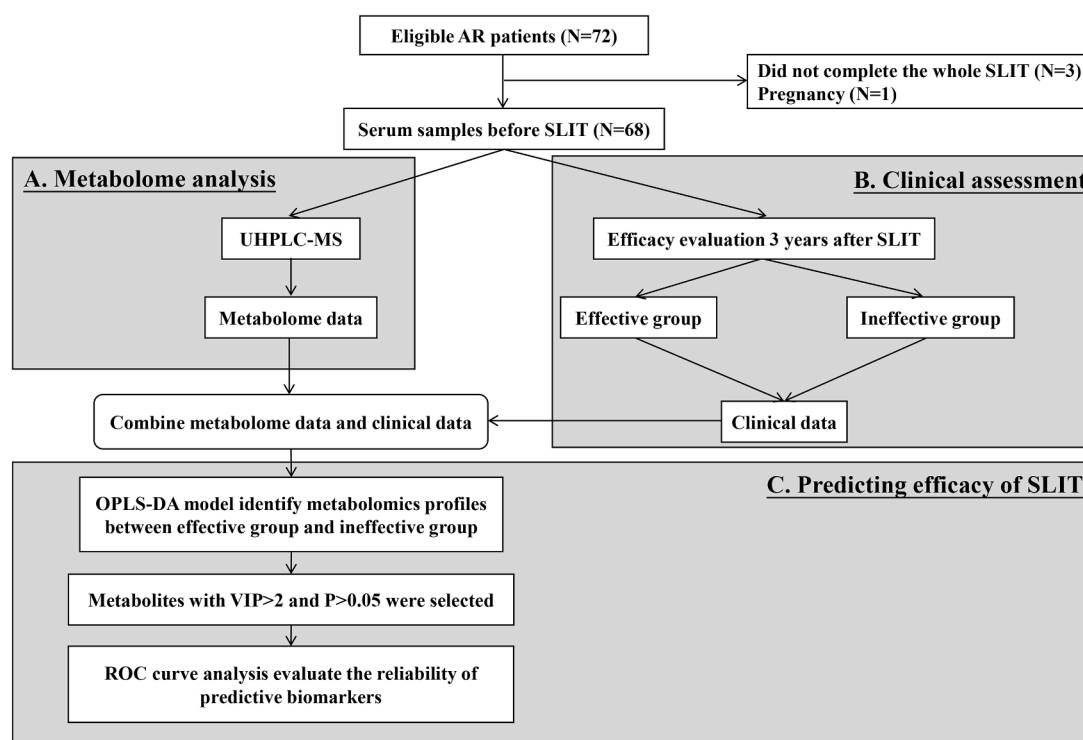


Fig. 1. An overview of study profile for identifying serum biomarkers predicting the efficacy of SLIT. (A) UHPLC-MS analysis was applied for explore the serum metabolic profiles. (B) AR patients were categorized at 3-years follow-up based on the reduction of SMS. (C) Metabolites were compared between effective group and ineffective group, and the discriminative abilities of metabolites were evaluated. SLIT, sublingual immunotherapy; UHPLC-MS, ultra-high performance liquid chromatography-mass spectrometry; AR, allergic rhinitis; SMS, symptom and medication score.

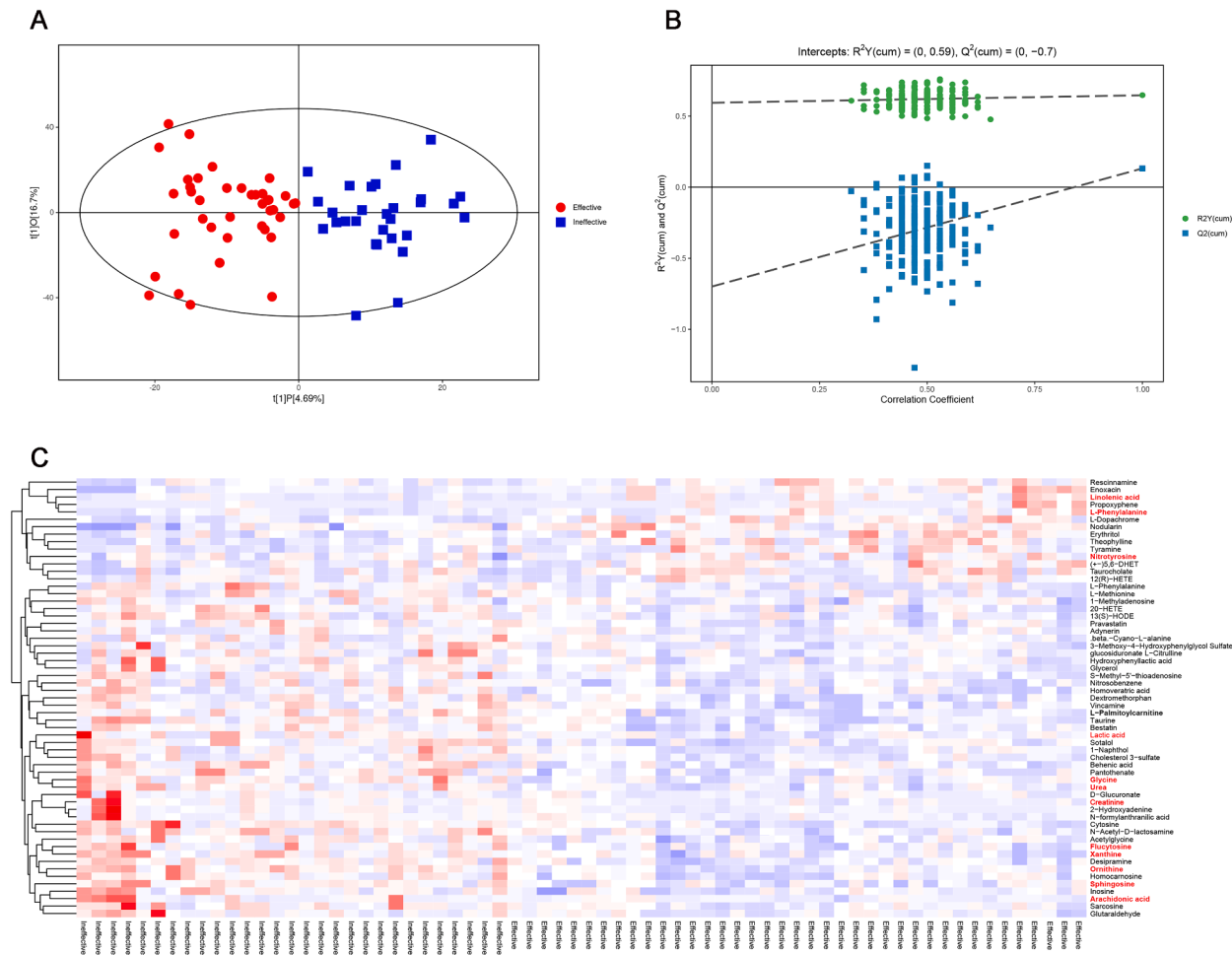


Fig. 2. Metabolomics analysis of serum samples form effective group and ineffective group. (A-B) OPLS-DA model and permutation test of the OPLS-DA model. (C) Heat map of the 59 discriminative metabolites between effective group and ineffective group. OPLS-DA, orthogonal partial least square-discriminate analysis.

analyses were performed using SPSS statistics software version19.0 (IBM, Chicago, IL, USA), and figures were plotted using GraphPad Prism 7.0 (Software Inc. La Jolla, CA, USA), and P values < 0.05 were considered statistically significant.

3. Results

3.1. Demographics and clinical characteristics of participants

In total, 68 patients completed the whole course of SLIT and were included in the present study. SLIT was effective in 39 patients (effective

group) and ineffective in 29 patients (ineffective group) at 3-years follow-up, and the overall effective rate was 57.4%. During the follow-up, 7 (10.3%) patients complained local adverse reactions, including 5 (7.4%) cases pruritus and swelling of mouth, tongue or eye, 1 (1.5%) case nose bleeding, and 1 (1.5%) case aggravating rhinitis, and no major systemic adverse reactions was reported. No significant pre-treatment differences were found in sex, age, BMI, disease duration, serum total IgE, baseline TNSS, VAS, MS and SMS between two groups (P > 0.05) (Table 1).

Table 2
Major metabolites contributing to predicting the efficacy of SLIT (VIP > 2).

Metabolites	VIP	P-value	Regulation	FC	Metabolism pathways
Lactic acid	2.63	<0.001	up	3.12	Glycolysis and pyruvate metabolism
Ornithine	2.57	0.012	up	2.37	Arginine and proline metabolism
Linolenic acid	2.53	0.006	down	0.32	Fatty acid metabolism
Flucytosine	2.42	0.020	up	2.09	Pyrimidine metabolism
Glycine	2.37	0.008	up	1.96	Glycine, serine and threonine metabolism
Creatinine	2.31	<0.001	up	1.87	Arginine and proline metabolism
L-Phenylalanine	2.28	0.008	down	0.48	Phenylalanine metabolism
Xanthine	2.25	<0.001	up	2.11	Purine metabolism
Nitrotyrosine	2.16	0.024	down	0.59	Tyrosine metabolism
Arachidonic acid	2.10	<0.001	up	2.20	Fatty acid metabolism
Urea	2.04	<0.001	up	1.88	Urea cycle
Sphingosine	2.01	<0.001	up	2.03	Sphingolipid metabolism

SLIT, sublingual immunotherapy; VIP, variable importance for project; FC, fold change.

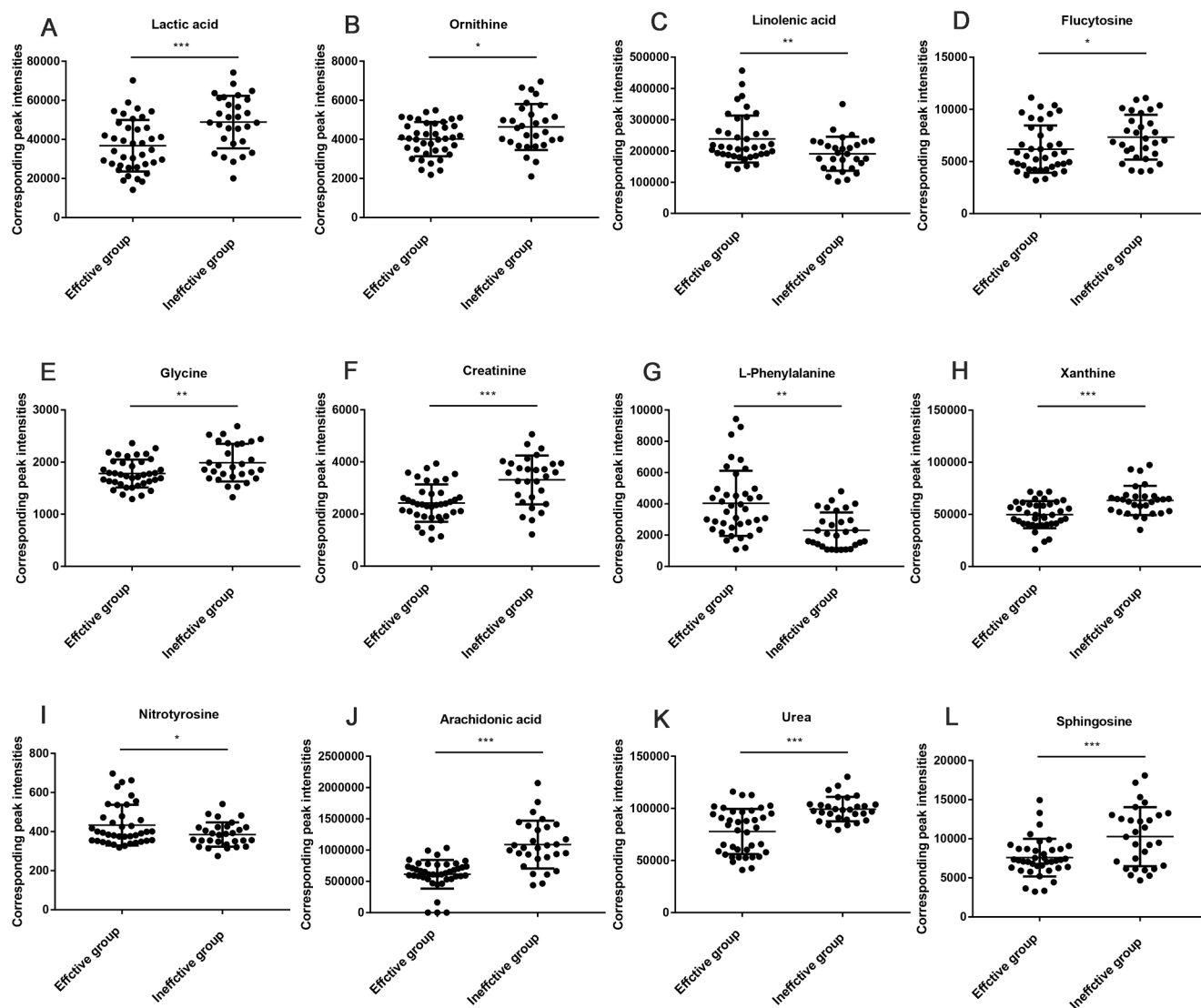


Fig. 3. Twelve most discriminant metabolites in their relative levels in effective group and ineffective group. Mann–Whitney *U* test was used for the statistical analysis. **P* < 0.05, ***P* < 0.001, ****P* < 0.001.

3.2. Distinct clustering of metabolites for effective group vs ineffective group

In the present study, we utilized UHPLC-MS to explore serum metabolites predictive of SLIT response. An overview of study profile was displayed in the Fig. 1. A total of 539 metabolites were obtained, and 197 of which were identified as known substances. Using these 197 known metabolites, OPLS-DA model showed a clear and distinctive clustering between the serum of effective group and ineffective group (Fig. 2A), indicating that some metabolites contributed to discriminate effective patients from ineffective patients. The permutation analysis exhibited that permuted *R*²_Y were 0.59 and permuted *Q*² were −0.7 (Fig. 2B), which suggested that the model fitting and prediction were valid.

3.3. Screening potential biomarkers for predicting SLIT efficacy

Based on the VIP value of metabolites in the OPLS-DA model and *t*-test discriminating (VIP > 1 and *P* < 0.05), we found 59 metabolites exhibited different accumulation patterns, including 45 up-regulated metabolites and 14 down-regulated metabolites in the ineffective group, which were visualized in a plot (Fig. 2C). Based on the heat map

results, the distribution of 59 differential metabolites could be divided visually between effective group and ineffective group. In order to explore more reliable metabolites that contributed to the efficacy prediction, we applied threshold of 2 for VIP. Ultimately, 12 metabolites were identified and listed in the Table 2, 9 of which were elevated in the ineffective group (lactic acid, ornithine, flucytosine, glycine, creatinine, xanthine, arachidonic acid, urea and sphingosine) and 3 of which were decreased in the ineffective group (linolenic acid, L-phenylalanine and nitrotyrosine). The relative levels of these remarkable biomarkers in the serum of the two groups were compared in the Fig. 3.

3.4. Receiver operating characteristic curve and metabolic pathway analysis

ROC curve analysis was conducted to evaluate the performance of above 12 remarkable biomarkers. In order to guarantee the reliability of potential biomarkers, the area under the curve (AUC) value of each metabolite should be higher than 0.7. The results showed that lactic acid, ornithine, linolenic acid, creatinine, arachidonic acid and sphingosine exhibited good performance in predicting the efficacy of SLIT (Fig. 4). In addition, significantly discriminative metabolites between two groups were subjected to pathway analysis using MetaboAnalyst to

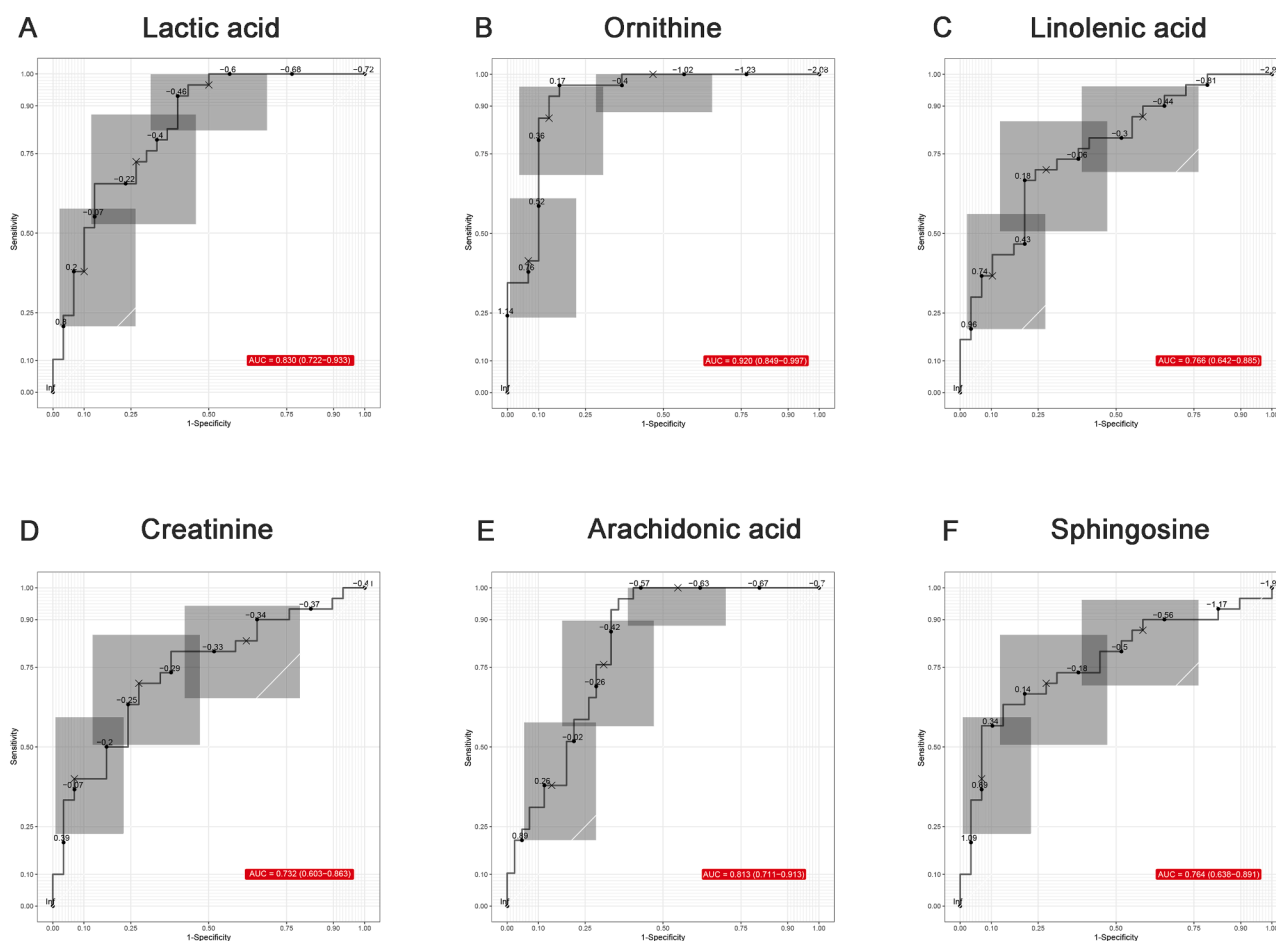


Fig. 4. The typical ROC curve plots of potential biomarkers with high-performance prediction, metabolites with an AUC value > 0.7 were displayed. ROC, receiver operating characteristics.

explore the most impacted pathways, and bubble plot shown that glycolysis and pyruvate metabolism, arginine and proline metabolism and fatty acid metabolism were the major affected metabolism pathways (Fig. 5).

4. Discussion

To the best of our knowledge, the present study is the first report to describe a novel application of metabolomics analysis in measuring serum metabolites of AR patients and identifying potential biomarkers for predicting the efficacy of SLIT prior to treatment delivery. Our results showed that effective group exhibited discriminative serum metabolites and metabolic pathways in comparison with ineffective group. We also identified 6 metabolites with high sensitivity and high specificity that contributed to discriminate the effective group from ineffective group, suggesting that these biomarkers were useful for predicting the efficacy of SLIT. These results indicated that serum metabolomics was useful and easily applied by allergy specialists to select patients who might benefit from SLIT and better personalize this widely used treatment.

Metabolomics is an advanced systematic biological technique which has been widely used in the early screening diagnosis and prognosis of diseases, identifying new drug target and monitor therapeutic effects [10,11]. As metabolites are the most downstream products of cellular metabolism, metabolomics analysis will provide valuable information about subtle changes in the biosystem at the cellular level [11,29]. In addition, metabolomics is a cost-effective, non-invasive and convenient omics approach, it is easy to be translated into clinical practice [9,30]. Previous studies reported that metabolomics was extensively applied to

explore metabolic changes in allergic diseases, and it was demonstrated to be an effective approach to develop biomarkers for diagnosis and predicting treatment [12,31]. Kowal et al [31] employed metabolomics to analyzed the serum metabolites and found that the sphingolipid metabolism was altered and associated with asthma phenotype in asthma patients. In another study, Crestani and colleagues applied untargeted metabolomics and identified a disease-specific metabolomics profile in the serum of food allergy children [32]. However, there were limited work illuminating the underlying metabolic processes and pathways associated with AR, and no study employing metabolomics to predict the efficacy of SLIT.

In our study, we included 68 AR patients who were assigned to receive SLIT, and categorize them into effective group and ineffective group based on their response to treatment. We utilized UHPLC-MS analysis to study the difference of metabolic profiles between two groups, and found that effective group exhibited distinctive serum metabolomics signatures in comparison with ineffective group. In order to identify more reliable and accurate metabolites that contributed to the prediction of the efficacy of SLIT, metabolites with a VIP > 2 and AUC > 0.7 were selected. Based on these evaluations, lactic acid, ornithine, linolenic acid, creatinine, arachidonic acid and sphingosine were selected as predictive biomarker candidates, and they were mainly involved in the glycolysis and pyruvate metabolism, arginine and proline metabolism and fatty acid metabolism, which were closely associated with immune response and immune tolerance.

Our results showed that lactic acid levels were elevated in the serum of AR patients who did not respond to SLIT. Lactic acid, an important component of glycolysis and pyruvate metabolism, is one of the most

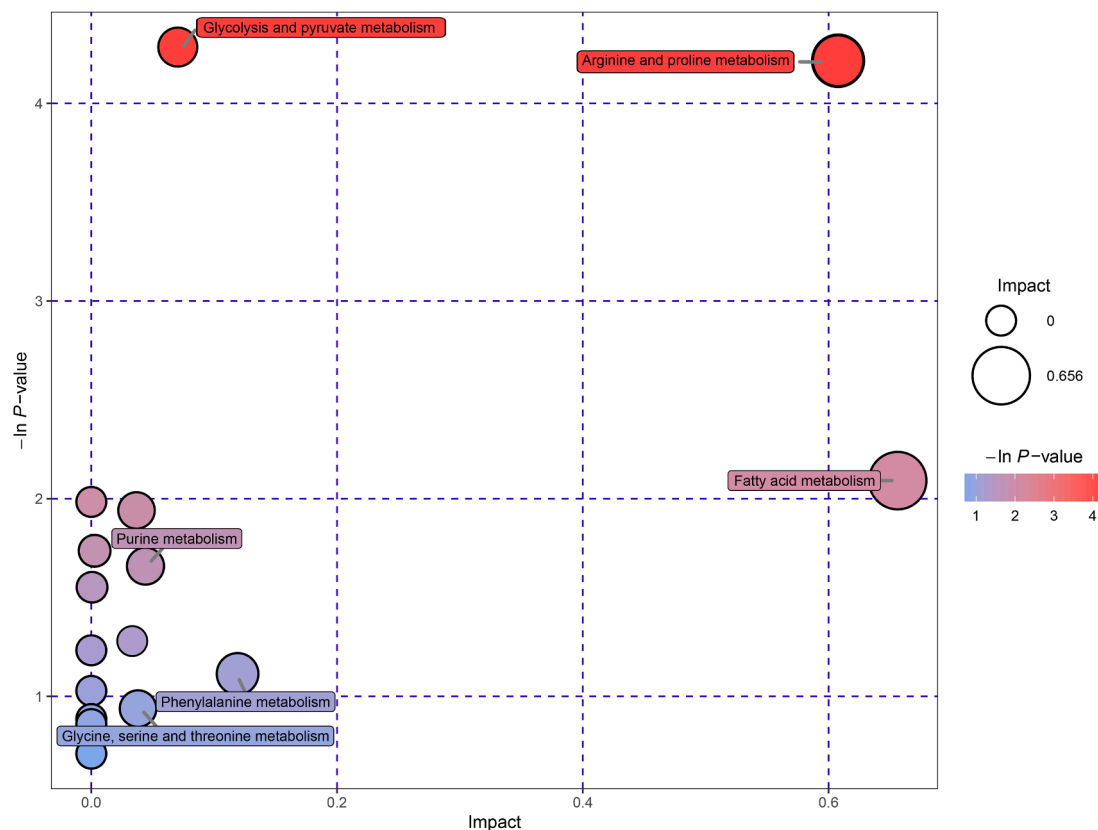


Fig. 5. A systemic view of altered metabolic pathways between effective group and ineffective group.

enriched by-products of cellular metabolism in bones, muscles, brain and erythrocytes, metabolized by the liver, and discharged by the kidneys. It is one of most important metabolic indications in proliferating cells, especially in inflamed tissues and tumors [33]. Accordingly, the lactic acid concentration in the peripheral blood can reflect the tissue oxygen supply and metabolism, especially during hypoxia, inflammation and tumor conditions [34]. Previous studies demonstrated that elevated serum lactic acid might induce the exacerbation of the inflammatory response in chronic inflammatory and allergic diseases like rheumatoid arthritis and asthma [35]. A recent study reported that the increased levels of lactic acid enhanced the production of IL-23 and IL-17 by CD4 + T cells, and lactic acid acted as a pro-inflammatory signal in several inflammatory disorders [36]. In another study, Pucino et al found that lactic acid and lactate accumulated at the site of chronic inflammation and aggravated the disease severity of rheumatoid arthritis by inducing CD4 + T Cell metabolic reprogramming [37]. Accumulating evidence highlighted that lactic acid was necessary and sufficient to induce macrophages polarization to a tumor-promoting state and immune response [33,36]. Therefore, we have reasons to believe that lactic acid may act a pivotal role in the AR, and can serve as a candidate biomarker for predicting the efficacy of SLIT.

Previous publications reported that arginine metabolism was pivotal in 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 play an important role in several inflammatory diseases [38]. In addition, arginine metabolism was demonstrated to be important in the nitric oxide (NO) synthesis and integrally links to cellular respiration, metabolism, and immune response [39]. NO is synthesized from L-arginine by NO synthase, and it is believed to be pivotal in the eosinophilic inflammatory response and act a biomarker of nasal inflammation in AR patients [40,41]. Studies reported immunotherapy could attenuate both upper and lower airway immune response and reduce the production of nasal NO and fractional

exhaled NO via regulating the arginine and proline metabolism [40]. In a recent study, researchers demonstrated arginine metabolism was significantly regulated in the asthma patients, and the elevated levels of arginine and its downstream metabolite, including ornithine, citrulline, creatine, creatinine and sarcosine, promoted the inflammatory response and aggravated asthma symptoms [42]. Here we firstly reported that ornithine and creatinine concentrations were higher in the serum of ineffective group than those in the effective group, which indicated that arginine and proline metabolism pathway was significantly perturbed and might be involved in the mechanisms of SLIT in AR. However, the underlying association is unknown and requires further exploration.

Linolenic acid and arachidonic acid are the common polyunsaturated fatty acids, and they are proven to be important in the modulation of immune responses in allergic and inflammatory diseases [43]. Previous studies showed that linolenic acid exhibited immunosuppressive effects through reducing the proliferation and activation of T cells in a dose-dependent manner, modifying mast cell functions and suppress its activation and secretion, and activating both autophagy and anti-oxidation in a synergistic feedback loop [44,45]. Therefore, linolenic acid greatly aid in the prevention and treatment of multiple inflammatory diseases. However, arachidonic acid was demonstrated to be a pro-inflammatory molecule which could be metabolized into various eicosanoids and lipid mediators participating in inflammation and immune response [46]. A recent study found that arachidonic acid metabolism was abnormally activated and promoted immune response in the AR patients [30]. In the present study, we found that the serum levels of linolenic acid were lower and arachidonic acid were higher in the ineffective group than in the effective group, and these metabolites exhibited good reliability for predicting the efficacy of SLIT. However, the biological functions of these biomarkers and their association with the mechanisms of SLIT need further exploration.

Our results also provide evidence for the sphingolipids metabolism alteration is involved in the physiopathologic mechanisms of AR.

Sphingolipids are ubiquitous components of the cell membrane and control crucial cellular functions such as the cell cycle, senescence, inflammation, apoptosis and cell migration [47]. Multiple studies have demonstrated that altered sphingolipid metabolism with an accumulation of sphingolipids acted an important role in cell growth, inflammation and immune response [48]. Recently, growing evidence showed that sphingolipid metabolism played a crucial role in the occurrence and development of allergic diseases, including asthma, food allergy and atopic dermatitis [49,50]. Sphingosine, a composition of sphingolipid, is of particular interest due to its biological functions, such as regulating immune cell proliferation, differentiation [51]. In a recent study, Toncic et al [49] found that the levels of sphingosine were elevated in the stratum corneum of atopic dermatitis, and associated with disease severity and local cytokine concentrations. Kowal et al [31] analyzed serum metabolites from asthma patients, and observed that sphingosine levels were significantly increased and associated with asthma phenotype. Here, we found that the serum levels of sphingosine were higher in the AR patients who did not respond to SLIT, suggesting that sphingosine may be a candidate biomarker for choosing SLIT.

Our study has some limitations which may affect the reported findings. First, the numbers of recruited participants are limited, and we did not validate the predictive power of these biomarkers by conducting other cohort studies. Second, all patients are recruited from single-center with the same ethnicity and region, which might limit their generalization. Last, there is a wide variation in evaluating the efficacy of SLIT among previous reports, and no consensus currently exists regarding the evaluation criterion, this may limit the applicability of our findings.

In conclusion, this study is the first prospective study using metabolic biomarkers to predict the efficacy of SLIT in the AR patients. By metabolomics analysis, we identified lactic acid, ornithine, linolenic acid, creatinine, arachidonic acid and sphingosine can accurately predict the efficacy of SLIT in AR patients. The discriminative metabolites and related metabolic pathways contributed to better understand the mechanisms of SLIT in AR patients. Large clinical trials are required to further validate the predictive abilities of these metabolites before they were used in clinical practice.

Ethics and consent statements

The present study was approved by ethical committee of Xiangya Hospital of Central South University. All participants provided signed informed consent.

Disclosure

The authors declare no conflict of interest in preparing this article.

CRediT authorship contribution statement

Shaobing Xie: Writing - original draft. **Sijie Jiang:** Writing - review & editing. **Hua Zhang:** Data curation. **Fengjun Wang:** Resources. **Yongzhen Liu:** Data curation. **Yongchuan She:** Investigation. **Qiancheng Jing:** Methodology, Investigation. **Kelei Gao:** Visualization, Validation. **Ruohao Fan:** Methodology. **Shumin Xie:** Software. **Zhihai Xie:** Conceptualization, Writing - review & editing. **Weihong Jiang:** Supervision, Writing - review & editing.

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