Metabolomic analysis of synovial fluids from rheumatoid arthritis patients using quasi-targeted liquid chromatography-mass spectrometry/mass spectrometry

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Abstract

Objective

Synovial fluid (SF) accumulates extensively in joints of individuals with rheumatoid arthritis (RA), which reflects the pathological state of the synovium and disease activity. This study applied quasi-targeted liquid chromatography-mass spectrometry/mass spectrometry, an advanced metabolomics technique, to find characteristic metabolisms in RA.

Methods

SF samples from the patients (n=20) were collected and examined using the metabolomic technique. SF samples from patients with osteoarthritis (OA) (n=20) were used as controls.

Results

Four hundred and seventy-nine variable metabolites were detected, and 250 of these metabolites were identified by searching the Human Metabolome Database (HMDB) and a self-constructed information list of possible metabolites. S-plot and volcano plot analysis detected 22 metabolites with differential levels in RA SF compared with those in OA SF. With these 22 candidate metabolites, pathway analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database detected upregulation of pyrimidine metabolism and purine metabolism, and downregulation of fatty acid biosynthesis and unsaturated fatty acid biosynthesis in RA SF. Receiver operating characteristic (ROC) analysis and logistic regression models detected increased levels of guaiacol, naringenin, phenylpropanolamine and vanillylmandelic acid in RA SF. Furthermore, the naringenin level showed positive correlation with rheumatic factor (RF) and anti-cyclic citrillinated peptides (anti-CCP) levels.

Conclusion

Our study suggests disturbed pyrimidine metabolism, purine metabolism, fatty acid biosynthesis and unsaturated fatty acid biosynthesis, as well as increased naringenin level, are characteristic metabolisms in RA.

Key words

fatty acid biosynthesis, mass spectrometry, metabolomics, naringenin, liquid chromatography, purine metabolism, pyrimidine metabolism, rheumatoid arthritis, synovial fluid, unsaturated fatty acid biosynthesis

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease with persistent synovitis, systemic inflammation and autoantibodies that can cause joint damage and disability (1). Synovial fluid (SF) extensively accumulates in synovial junction of individuals with RA. SF is the main source of nutrition and lubrication for articular cartilage. SF directly contacts the joint, therefore more directly reflecting the pathological state of the synovium and disease activity than the blood samples. Thus, investigating metabolite changes in SF rather than in serum or plasma may be very helpful to understand the pathogenesis and identify biological markers in RA (2).

Metabolomics can detect changes in small molecular metabolites with high throughput, provide information on the unique characteristics and physiological states of biological systems, and thus help to identify new diagnoses, prognoses and therapeutic intervention targets in human diseases. Metabolomics can be divided into targeted analysis and non-targeted analysis. Non-targeted methods based on time-of-flight (TOF) mass spectrometry have the advantages of high analysis flux and abundant data information but have defects in data stability, repeatability and quantitative linear range (3). Targeted analysis was designed based on triple-quadrupole (TQ) mass spectrometry multiple-reaction monitoring (MRM) technology and can address the deficiency of non-targeted methods, but there are still some deficiencies, such as a limited number of targets, and the establishment of the method depends on standard (STD) substances, which is not suitable for large-scale metabolic profile analysis (4). The quasi-targeted technical scheme combines high-throughput and unbiased metabolite information acquisition of non-targeted analysis with high-specificity detection and accurate quantification of targeted analysis. Therefore, this new technology that started in recent years realises the simultaneous detection of multiple known and unknown metabolite ion targets in a sample (5). This technique not only ensures the coverage and detection sensitivity of metabolome information but also significantly improves the indices such as linear range and repeatability of data and ensures subsequent marker discovery and verification based on metabolome data (6, 7).

Nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS)-based metabolomics had been applied to search for biological markers in the SF of individuals with RA (8-11). Liquid chromatography-mass spectrometry (LC-MS)-based non-targeted/targeted metabolomics has been used for the discovery of biomarkers in the SF of individuals with RA or osteoarthritis (OA) (12-14). Non-targeted ultraperformance liquid chromatography quadruple TOF mass spectrometry had been also applied to analyse SF from 10 patients with RA (15). Recently, However, quasi-targeted LC-MS/MS metabolomics, an advanced metabolomics technique, has not been applied to screen RA markers in SF. The present study used metabolomics to analyse changes in small molecular metabolites with high throughput in SF samples from individuals with RA.

In this study, SF samples were aspirated from 20 patients with RA and 20 patients with OA, which were analysed by LC-MS/MS technique. The purpose of this study was to determine the metabolic characteristics using new a metabolomic technique, analyse the relationship between differential metabolism and clinical variables, and screen possible biomarkers of RA.

Methods

Collection of SFs

RA was diagnosed according to the 1987 American College of Rheumatology (ACR) classification criteria (16) and the 2010 ACR/European League against Rheumatism (ACR/EULAR) classification criteria (17). OA was diagnosed according to clinical criteria and the Kellgren and Lawrence scale (18). SF samples were obtained during knee arthroscopy from RA patients (n=20) and OA patients (n=20) at The Affiliated Hospital of Qingdao University (China) in Qingdao, China. The use of SF for research was performed under the guidance of the Helsinki Dec-

laration and approved by the Ethics Committee of The Affiliated Hospital of Qingdao University (20180901). Informed consent was obtained from all patients who provided the samples. The samples were kept at -80 °C before sample preparation. The clinical information is provided in Supplementary Table S1.

Sample preparation

OA and RA SF from knee joints were aspirated into heparinised tubes and were then centrifuged at >2000g for 5 min. The cell-free SF was decanted and frozen at -20°C. A 150-μL aliquot of SF samples was added to 430 μL of methanol and 20 μL of 2-chloro-l-phenylalanine (0.3 mg/mL) was added. The mixtures were vortexed for 1 min, ultrasonicated at ice-cold temperature for 5 min, stored at -20 °C for 30 min, and centrifuged at 13,000 rpm for 10 min at 4 °C. A 450-μL aliquot of the supernatant was evaporated in a ryocentrifugal concentrator, and 250 μL of an ice-cold mixture of methanol and water (1/1, v/v) was added. The mixtures were vortexed for 30 s and ultrasonicated at ice-cold temperature for 2 min. A 200-μL aliquot of chloroform was added, and the mixtures were vortexed for 1 min, stored at -20°C for 20 min, and centrifuged at 13,000 rpm for 10 min at 4°C. A 180-μL supernatant aliquot was transferred to a liquid chromatography (LC) vial and stored at -20°C until LC-MS analysis.

SF metabolomic profiling

Randomised and sex-matched SF samples from RA (n=20) and OA (n=20) patients were used for metabolomic profiling by an ACQUITY UPLC I-Class system (Waters Corporation, Milford, USA) coupled with a VION IMS QTOF mass spectrometer (Waters Corporation, Milford, USA). The LC flow rate was 0.4 mL/min, and solvents A (a mixture of acetonitrile and 10 mM ammonium acetate (pH=9) (90/10%, v/v)) and B (10mM ammonium acetate (pH=9) were used. The injection volume was 3 μL, and the column temperature was 45°C. The solvent B elution gradient was as follows: 0 min, 5%; 1.5 min, 25%; 10 min, 90%; 13 min, 90%; 13.5 min, 5% and 14.5 min, 5%. Data acquisition was performed in full scan mode (m/z ranges from 50 to 1000), and the scan time was 0.1 s. The capillary voltage was 1.0 kV, and the sampling cone voltage was 40 V. The source temperature was 120°C. The desolation temperature was 550°C, and the desolation gas flow was 900 L/h. To

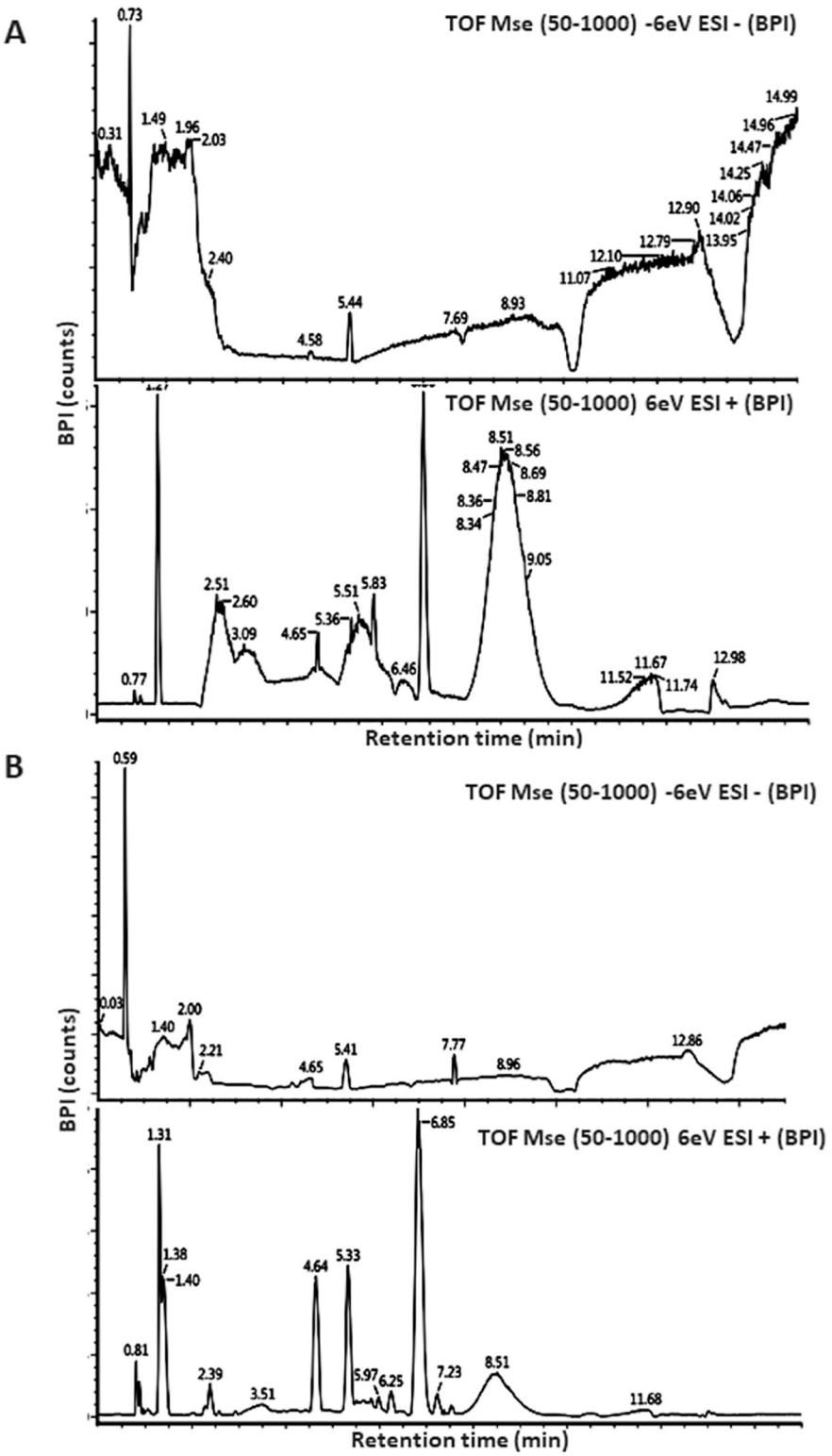

Fig. 1. Typical base peak intensity chromatograms of SF samples.
A: Typical base peak ion chromatograms of OA samples.
B: Typical base peak ion chromatograms of RA samples.
BPI: base peak ion.

check the stability of the system, quality control (QC) samples were injected at regular intervals (every 10 samples) throughout the analytical run.

Data preprocessing and statistical analysis

Progenesis QI v. 2.3 software (Waters Corporation, Milford, USA) was used for peak identification, identification of quasi-targeted metabolites, peak extraction, peak alignment and quantification. The precursor tolerance was 5 ppm, the product tolerance was 5 ppm and the production threshold was 5%. Identification of quasi-targeted metabolites was based on STD substances. The identification of other non-targeted metabolites was based on accurate mass number, secondary fragments and isotope distribution. Qualitative analysis was carried out using the HMDB database (http://www.hmdb.ca/). Positive and negative data were combined to obtain combined data, and the obtained untargeted data and quasi-targeted data were integrated into a data matrix. SIMCA software (v. 14.0, Umetrics, Umeå, Sweden) was used for orthogonal partial least squares-discriminant analysis (OPLS-DA). Differentially abundant metabolites were selected on the basis of variable importance in projection (VIP) from the OPLS-DA, *p*-values from a two-tailed Student's t-test ($p<0.05$), and fold change (FC >2 or FC <0.5) from a volcano map. Logistic regression and receiver operating characteristic (ROC) curves were used to evaluate the predictive accuracy of the models.

Statistical analysis

Two-tailed Student's t-test were used to compare the differences of metabolites data between rheumatoid arthritis patients and osteoarthritis patients. Chi-square test and Mann-Whitney U-test were used to compare the differences of clinical data between rheumatoid arthritis patients and osteoarthritis patients. Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad, USA) and SPSS software v. 21.0 (IBM, USA). Data were recorded by Mean and SD. $p<0.05$ were considered significant.

Results

Metabolomic profiling by LC-MS analysis

The samples of OA (n=20) and RA subjects (n=20) were analysed to identify metabolite biomarkers in RA SF using a quasi-metabolomic approach. Typical base peak ion chromatograms of the OA and RA groups are shown in Figure 1 (A-B). Positive ion mode detected 240 variables, and negative ion mode detected 239 variables. A total of 250 metabolites of these 479 variables were identified after searching the Human Metabolome Database (HMDB).

To provide evidence of good quality control, use of pooled QC (aliquot of each sample pooled together) showed the stability of the run and showed where they cluster together in PCA analysis (Fig. 2A). OPLS-DA, the most frequently used multivariate statistical method, was used to characterise metabolic disturbances. The quality of the OPLS-DA model was assessed by the R2Y and Q2, which can indicate the explanation ability and predictive capacity of a model (19). The R2Y and Q2 of the OPLS-DA model were 0.994 and 0.771, respectively, indicating that the model was stable and reliable. The OA score plots of OPLS-DA (red circle) were distributed in the P1 component direction, and the RA score plots (blue box) were separated in the P2 component direction, indicating that the model could discriminate metabolites between the OA and RA groups (Fig. 2B).

To discover potential differentially abundant metabolites, volcano plots were prepared by analysing FC and *p*-values of t-test results. The volcano plots were constructed by plotting the log of the FC between RA and OA samples on the x-axis (base 2) and the log of the *p*-value on the y-axis (base 10)**.** Compared with those in the OA group samples, the levels of phenylpropanolamine, vanillylmandelic acid, l-aspartyl-l-phenylalanine, tauroursodeoxycholic acid, quinolinic acid, guaiacol, naringenin, cytosine, 1-methylhistidine, paraxanthine, deoxyuridine, l-fucose, inosine, guanosine, alpha-n-phenylacetyl-l-glutamine, methionine sulfoxide, guanosine monophosphate and glutaric acid were significantly elevated in RA SF samples. Meanwhile, the levels of four metabolites, palmitoleic acid, l-3-phenyllactic acid, linoleic acid and n-acetyl-l-aspartic acid, were significantly decreased in the RA samples (Fig. 2C). To restrict the potential variables, those metabolites with features of FC >2 or <0.5 and $p<0.05$ were defined as significantly different. These 22 annotated metabolites among the 250 metabolites had significantly changed levels in RA SF (Fig. 2D), and the detailed information is also shown in Supplementary Table S2.

Pathway analysis of metabolic pathways

Metabolic pathways were analysed with these 22 verified differentially abundant metabolites with the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/KEGG/pathway.html). To find the key pathway most related to differentially abundant metabolites, a bubble chart of enrichment results and a KEGG enrichment histogram were prepared by analysing the *p*-value and matched metabolite number. Compared with those in OA samples, pyrimidine metabolism ($p=0.00395$), purine metabolism ($p=0.0136$), fatty acid biosynthesis ($p=0.0297$), and unsaturated fatty acid biosynthesis ($p=0.0356$) were strikingly disturbed in RA SF samples. Levels of cytosine and deoxyuridine in pyrimidine metabolism; inosine, guanosine and guanosine monophosphate levels in purine metabolism were significantly higher in RA than in OA, whereas levels of palmitoleic acid in fatty acid biosynthesis and linoleic acid in unsaturated fatty acid biosynthesis were significantly lower in RA than in OA (Fig. 3A-B).

Correlation between RA clinical indices and the validated metabolites

ROC analysis and logistic regression models were used to further examine the association between those 22 candidates and rheumatoid factor (RF) or anti-cyclic citrullinated (anti-CCP) levels with significantly changed levels in RA SF. By ROC analysis, an area under the curve (AUC) of the ROC >0.9 was considered excellent, 0.8–0.9

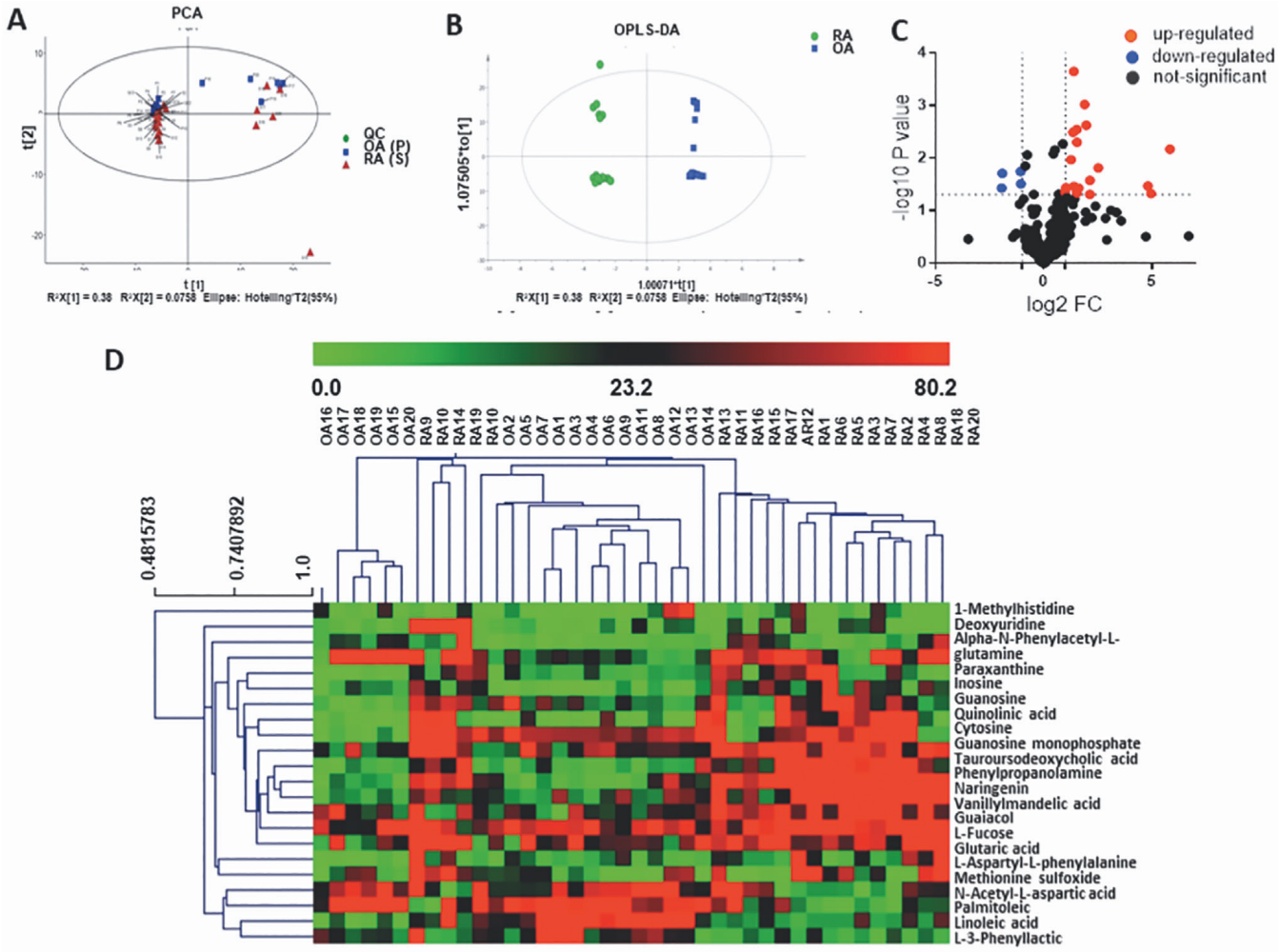

Fig. 2. Multivariate statistical analysis of SF samples.
A: PCA analysis of SF metabolome of OA (blue box), RA (red triangle) and QC (green circle).
B: Orthogonal partial least-squares-discriminant analysis (OPLS-DA) of SF metabolome of OA (blue box) and RA (green circle).
C: Volcano plots of statistical significance of metabolite changes between OA and RA. (p<0.05; FC >2).
D: Heat map of 22 discovered biomarkers in SF samples. FC: fold change.

very good, 0.7–0.8 good, 0.6–0.7 average, and <0.6 poor (20). Among the 22 candidates, AUC values of naringenin (AUC=0.9300, p<0.0001), phenylpropanolamine (AUC=0.8425, p=0.0002), vanillylmandelic acid (AUC=0.8225, p=0.0005) and guaiacol (AUC=0.8225, p=0.0005) were higher than 0.8, which was considered an excellent diagnostic test (Fig. 4 A-D), meanwhile AUC values of other targeted metabolites were <0.8 or p-value were >0.05 (Table I).

By logistic regression analysis, higher levels of naringenin, phenylpropanolamine, vanillylmandelic acid and guaiacol were found to be significantly associated with RA (odds ratio (OR) = 1.294 (1.073–1.561), 1.505 (1.142–1.984), 1.217 (1.065–1.390) and 1.023 (1.005–1.042), respectively) (Table I). Furthermore, levels of naringenin, phenylpropanolamine, vanillylmandelic acid and guaiacol were significantly higher in RA SF than in OA SF (p=0.0067, 0.0002, 0.0010 and 0.005, respectively). Using the optimal cut-off values, prediction accuracies of the above 4 metabolites were 85%, 50%, 70%, and 60% for RA, respectively (Suppl. Fig. S1A and S2A).

A strong correlation was detected for naringenin (R2=0.88) with RF value in RA SF, while weak correlations were detected for vanillylmandelic acid and guaiacol (R2=0.4 and 0.47) with the serum RF level. No correlation was detected for phenylpropanolamine with serum RF in RA SF (p=0.61) (Suppl. Fig. S1B and S2B). Additionally, a strong correlation was obtained for naringenin (R2=0.86) and weak correlations for VMA and guaiacol (R2=0.44 and 0.47) with serum anti-CCP content in RA SF. No correlation was observed for phenylpropanolamine with the serum anti-CCP level (p=0.66) (Suppl. Fig. S1C and S2C).

Discussion

To investigate distinctive metabolisms in SF as well as the relative pathogenesis in RA, this study performed quasi-targeted LC-MS/MS-based metabolomic analysis with SF samples from individuals with RA. The OPLS-DA model was used to characterise the metabolic disturbances. Of the 250 metabolites that we identified, 22 candidate metabolites in SF differed in VIP value, FC and p-value between RA and OA.

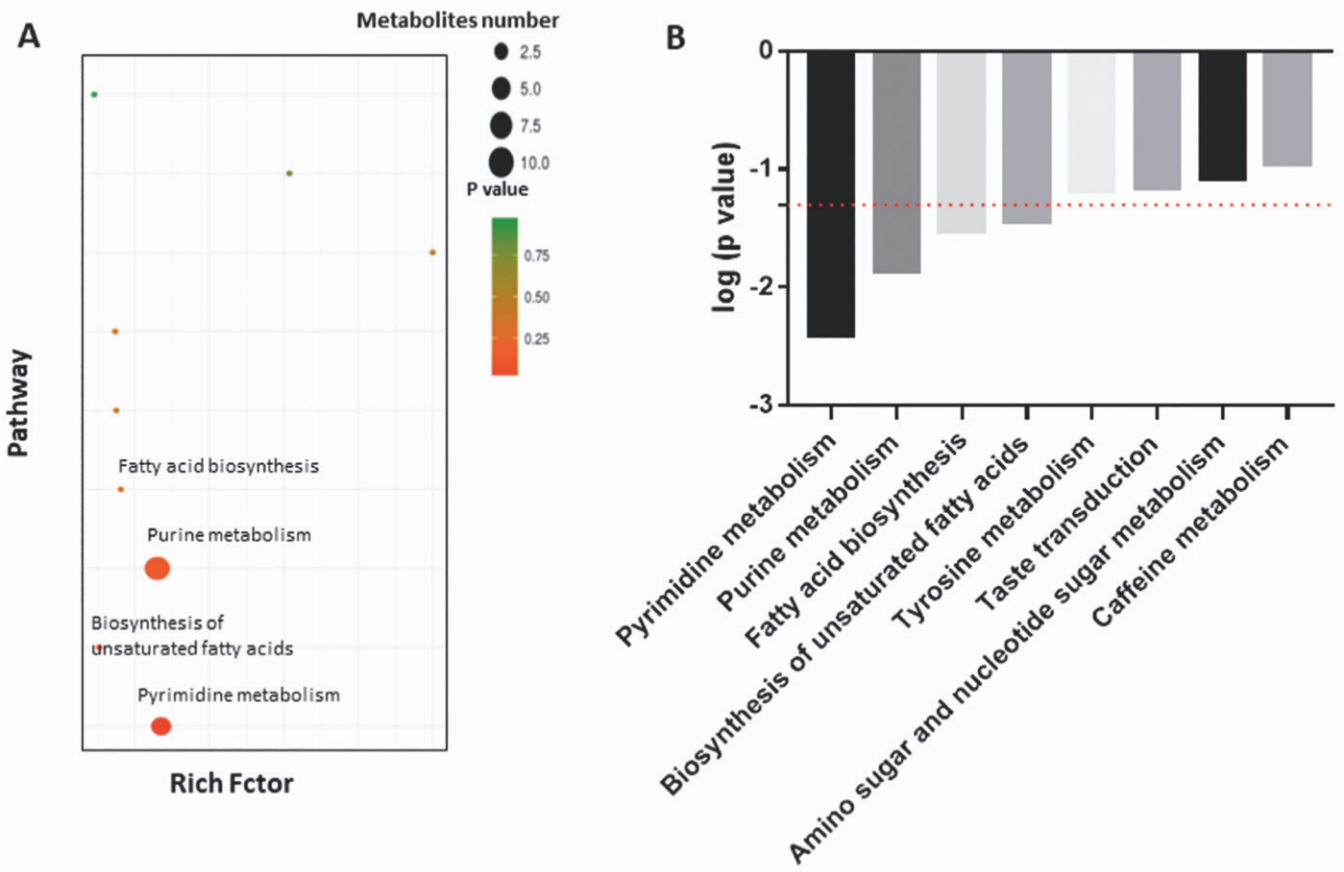

Fig. 3. Pathway analysis in RA with the verified differentially abundant metabolites. **A**: Bubble chart of enrichment map. **B**: KEGG enrichment histogram. When the top of the column was lower than the red line (p=0.05), the signal path represented was significant.

With these 22 candidate metabolites, pathway analysis demonstrated activation of pyrimidine metabolism and purine metabolism, and suppression of fatty acid biosynthesis and unsaturated fatty acid biosynthesis in RA SF. Pyrimidine and purine metabolism belong to nucleotide metabolism. Activated pyrimidine and purine metabolism had been noticed in RA, although there is no metabolomic evidence in human samples so far. Pharmacological studies show that methotrexate, a first-line drug for RA, can alleviate RA progression by inhibiting pyrimidine metabolism and purine metabolism (21-24); Leflunomide, a selective inhibitor of de novo pyrimidine synthesis to alter the metabolism of pyrimidines, has been successfully used for treating RA and psoriatic arthritis for many years (25, 26); Lapachol, a compound targeting pyrimidine metabolism, can ameliorate experimental autoimmune arthritis (27). A metabolomics study recently found altered purine, pyrimidine and pentose phosphate metabolisms in rat model with collagen II-induced arthritis and suggested that inhibition on the nucleic acid synthesis was very helpful to RA therapy (28). The above studies were consistent with our finding about upregulation of pyrimidine and purine metabolisms in RA.

Biosynthesis of fatty acids and unsaturated fatty acids plays an important role in RA (29-32). The present study detected decreased biosynthesis of fatty acids and unsaturated fatty acids in RA. This finding was also supported by the metabolomic study in CIA model (28), although there is no metabolomic evidence in human samples so far. Pharmacological studies showed that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), a representative substance of n-3 polyunsaturated fatty acids (n-3 PUFAs, omega-3), can ameliorate disease activity in patients with RA (27, 28, 33). A systematic review and meta-analysis found that intake of ω-3 polyunsaturated fatty acids improved RA therapy in patients (33). Monounsaturated fatty acids in the Mediterranean diet can suppress RA disease activity (34). A recent study demonstrated that omega unsaturated fatty acids (ω-UFAs) had anti-inflammatory effect on collagen-induced arthritis in DBA/1 Mice (35). The above studies were consistent with our finding about downregulation of fatty acids and unsaturated fatty acids in RA.

ROC analysis and logistic regression models were used to further examine the association between RA clinical indices and these 22 candidate metabolites. Phenylpropanolamine, guaiacol and vanillylmandelic acid, showed increased levels in RA SF. Phenylpropanolamine, a risk factor for hemorrhagic stroke, is commonly found in appetite suppressants and cough or cold remedies (36). However, the role of phenylpropanolamine in RA has not yet been reported. Guaiacol is a phenolic natural product that was isolated from guaiac resin and the oxidation of lignin. Guaiacol has

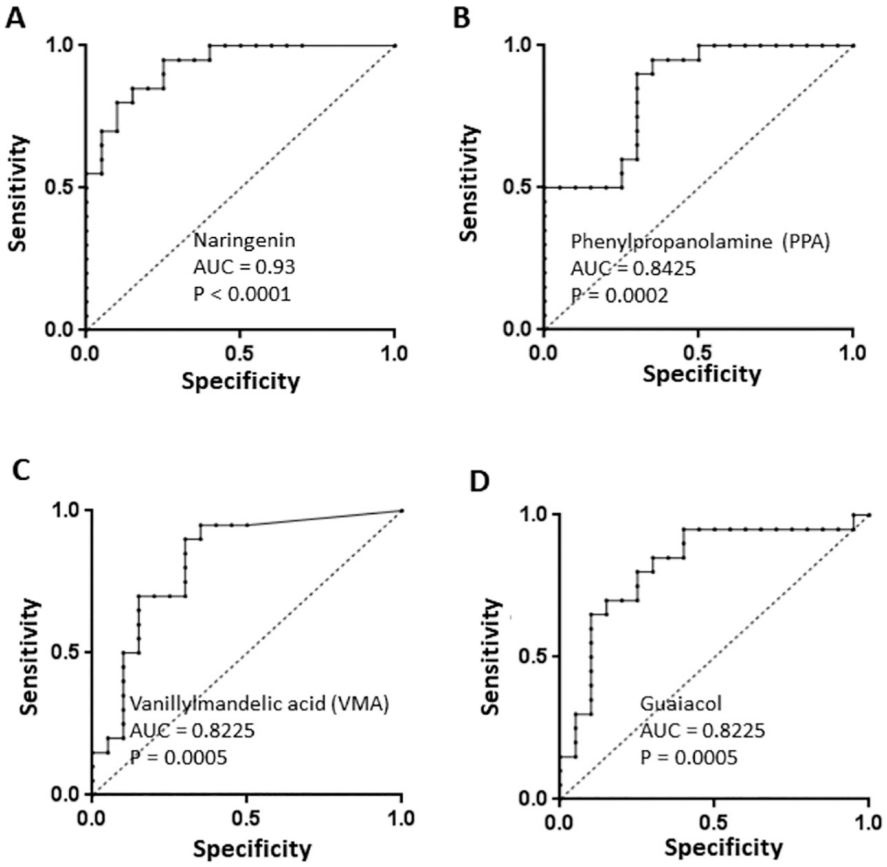

Fig. 4. ROC analysis and Logistic regression of 22 candidate metabolites in RA SF.
A: naringenin, **B**: phenylpropanolamine, **C**: vanillylmandelic acid; **D**: guaiacol.

Tab. I. ROC analysis and Logistic regression analysis of candidate metabolites.

Metabolites	AUC	*p*-value	OR (95%CI)	*p*-value
Phenylpropanolamine	0.842	0.0002	1.505 (1.142-1.984)	0.004
Vanillylmandelic acid	0.822	0.0005	1.217 (1.065-1.390)	0.004
L-Aspartyl-L-phenylalanine	0.767	0.0038	1.064 (1.014-1.117)	0.012
Tauroursodeoxycholic acid	0.765	0.0041	1.004 (1.001-1.006)	0.013
Quinolinic acid	0.736	0.0106	1.020 (1.004-1.037)	0.017
Guaiacol	0.822	0.0005	1.023 (1.005-1.042)	0.012
Naringenin	0.93	<0.0001	1.294 (1.073-1.561)	0.007
Cytosine	0.78	0.0024	1.030 (1.004-1.057)	0.025
1-Methylhistidine	0.655	0.0935	1.143 (0.993-1.095)	0.093
Palmitoleic acid	0.662	0.0787	0.991 (0.981-1.001)	0.065
L-3-Phenyllactic acid	0.79	0.0017	0.899 (0.824-0.980)	0.015
Paraxanthine	0.645	0.1167	1.023 (1.000-1.046)	0.049
Linoleic acid	0.617	0.2036	1.000 (0.999-1.000)	0.044
Deoxyuridine	0.64	0.1298	4.953 (0.34-70.94)	0.239
L-Fucose	0.755	0.0058	1.098 (1.000-1.205)	0.051
Inosine	0.627	0.1677	1.002 (1.000-1.004)	0.06
Guanosine	0.612	0.2235	1.006 (0.999-1.014)	0.082
N-Acetyl-L-aspartic acid	0.601	0.2733	0.947 (0.948-1.001)	0.06
α-N-Phenylacetyl-L-glutamine	0.695	0.0349	1.001 (1.000-1.003)	0.062
Methionine sulfoxide	0.705	0.0265	1.049 (0.994-1.108)	0.081
Guanosine monophosphate	0.637	0.1368	1.178 (0.994-1.171)	0.071
Glutaric acid	0.701	0.0294	1.226 (0.963-1.563)	0.099

long been used in clinical trials for RA treatment. As early as 1925, Kraiem *et al*. found that intramuscular injection of guaiacol dissolved in oil was satisfactory in nine cases of RA (37). Derivatives, cross-coupling of guaiacol and sinomenine were more potent than guaiacol for the treatment of RA (38). An increased level of guaiacol in our study could be due to medical residues of previous anti-inflammation treatment. Vanillylmandelic acid, one of the most important catecholamine metabolites, is commonly used in the diagnosis of pheochromocytoma. Urinary vanillylmandelic acid was decreased in adjuvant-induced arthritis (AIA) rats (39). In contrast, UPLC-LTQ/Orbitrap MS with untargeted metabolomics recently detected increased levels of vanillylmandelic acid in adjuvant-induced arthritis (AIA) rats (40), which was consistent with the results of our study. We suggest that phenylpropanolamine and vanillylmandelic acid could be possible risk factors for RA, although their role in RA needs further exploration.

The present study not only detected an increased level of naringenin in RA SF but also demonstrated a positive correlation of naringenin level with RF and anti-CCP levels in RA. Naringenin is an abundant flavonoid compound in daily food and drinks, such as fruits, vegetables, nuts, coffee, tea and wine, which can be more easily absorbed by intestines than other flavonoids (41). Naringenin is a well-known immunomodulator that possesses various biological activities, such as anti-inflammatory activity (42). Zhu *et al*. also found that naringenin can relieve inflammation in complete Freund's adjuvant-induced arthritis by regulating the Bax/Bcl-2 balance (43). On the other hand, naringenin significantly reduces lung metastases in mice with pulmonary fibrosis and increases their survival by improving the immunosuppressive environment through down-regulating transforming growth factor-beta1 (TGF-β1) and reducing regulatory T cells (Treg cells) (44). Naringenin also prevents TGF-β1 secretion from breast cancer and suppresses pulmonary metastasis (45). Many studies demonstrated that the numbers and function of Treg cells are impaired in the disease, but the reason is not entirely understood (46, 47). Thus, naringenin has possibility to promote RA by reducing the numbers of Treg cells. TGF-β1 induced $CD4^+$ T cells to differentiate into Treg cells (48, 49). Interestingly, naringenin has a similar chemical structure to oestrogen and has

an oestrogen-like effect (50). RA has a high incidence rate in women, suggesting that oestrogen may play a role in the development of the disease (51, 52). Our study emphasises that naringenin could be a risk factor for RA occurrence.

Some groups had conducted metabolic analysis with RA synovial fluids, although they did not apply quasi-targeted LC-MS/MS metabolomics. Kim *et al*. analysed SF samples from 38 patients with RA, ankylosing spondylitis, Behçet's disease or gout using gas chromatography/time-of-flight mass spectrometry (GC/TOF MS). A total of 105 metabolites were identified from these SF samples. These metabolites were found to be associated with the urea and tricarboxylic acid cycle as well as fatty acid and amino acid metabolism (8). Yang *et al*. screened different metabolites in SF samples from 25 RA patients and 10 normal subjects using GC/TOF MS analysis. A subset of 58 metabolites was identified. The concentration of glucose was decreased, and the concentration of lactic acid was increased in the synovial fluid of RA patients than normal subjects (9). Anderson *et al*. identified different metabolites in SF of 10 OA and 14 RA patients using 1H nuclear magnetic resonance (NMR). A total of 32 metabolites showed significantly different between OA and RA SF, including amino acids, saccharides, nucleotides and soluble lipids. They found that metabolites of glycolysis and the tricarboxylic acid cycle were lower in RA compared to OA (10). Ahn *et al*. investigated metabolic perturbation in SF from 48 RA patients using gas chromatography/time-of-flight mass spectrometry (GC/TOF MS). They found the significant correlation between DAS28-ESR(3) value and the intensities of 12 metabolites. The intensities of glycocyamine and indol-3-lactate positively correlated with DAS28-ESR(3) value, and β-alanine, asparagine, citrate, cyano-L-alanine, leucine, nicotinamide, citrulline, methionine, oxoproline, and salicylaldehyde negatively correlated with DAS28-ESR(3) value. They further found 15 pathways that were significantly associated with disease activity in RA and that the higher the disease activity, the more amino acid metabolic processes were affected (11). Carlson *et al*. analysed the metabolomic profiles of synovial fluid from RA (n=3) and healthy (n=5) SF samples by LC-MS analysis. A total of 162 metabolites showed significantly different between diseased and control. Pathways upregulated with disease included ibuprofen metabolism, glucocorticoid and mineralocorticoid metabolism, alpha-linolenic acid metabolism, and steroid hormone biosynthesis, and pathways downregulated with disease included purine and pyrimidine metabolism, biological oxidations, arginine and proline metabolism, the citrulline-nitric oxide cycle, and glutathione metabolism (12). Combined with results of ours, significant alternation in purine and pyrimidine metabolism, fatty acid and amino acid metabolism, glycolysis and the tricarboxylic acid cycle were detected by the studies using metabolomics method. In summary, the present study detected activation of pyrimidine metabolism and purine metabolism, and suppression of fatty acid biosynthesis and unsaturated fatty acid biosynthesis in RA SF. The study also detected increased levels of phenylpropanolamine, guaiacol, vanillylmandelic acid and naringenin. Furthermore, the naringenin level has a significantly positive correlation with RF and anti-CCP levels in RA. The above findings may be helpful to understand the changed metabolism and pathogenesis of RA. This study also suggests that the application of quasi-targeted LC-MS/MS can detect more metabolic pathways than non-targeted ultraperformance LC quadruple TOF mass spectrometry in an RA study.

Acknowledgements

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