Urinary Metabolomics as a Potentially Novel Diagnostic and Stratification Tool for Knee Osteoarthritis

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Abstract:

Metabolomics has been used as a tool in disease diagnosis and phenotype prediction. A urinary metabolomic study based on GC–MS in combination with multivariate statistics was used here to classify between knee osteoarthritis (OA) and healthy controls. OPLS-DA of the spectral data showed distinct metabolic profile variations between OA patients and healthy controls and between two OA phenotypes. Differential metabolites reveal up-regulated TCA cycle associated with OA and histamine metabolism disorders accompanied with knee effusion symptoms. This metabolomic method is potentially applicable as a novel strategy for OA diagnosis and patient stratification.

Article:

INTRODUCTION

Osteoarthritis (OA) of knee joint is one of the most prevalent musculoskeletal arthropathy of the elderly worldwide, and is characterized by the progressive destruction of knee articular cartilage and concomitant change in subchondral bone that is frequently associated with chronic pain and disability (Hinman and Crossley 2007). It is currently estimated that approximately 46 million people in the United States have some type of arthritis, which is estimated to rise 60 million by 2030, and, that the most common type of arthritis is OA which currently affects in excess of 27 million people in USA (Murphy et al. 2008). OA is a slowly evolving process in which initiation of joint damage may be followed by further cartilage degradation as well as an attempted repair response (Huber et al. 2000). The exact pathogenesis of OA is still unknown, although genetic and environmental factors, including age, gender, obesity, ethnicity, inherited susceptibility, sports and physical activity, and muscle weakness, have been shown to predispose to the development of OA (Garstang and Stitik 2006).

There are different clinical phenotypes of OA, including OA associated with synovitis, OA associated with osteophyma. OA associated with synovitis is the type of inflammatory OA, and a number of studies have demonstrated an association between synovitis and progression of

cartilage structural changes (Pelletier et al. 2001; Loeser 2006). Another sub-clinical phenotype is OA associated with osteoporosis (Dequeker 1985; Hart et al. 1994). The pathological characteristics of these OA phenotypes differ, as do their corresponding therapeutic methods, so accurate diagnosis of OA and stratification between OA phenotypes are of great importance for optimized treatment and prognosis of OA. The current diagnostic methods of OA progression are mainly based on radiographic findings. However, radiography only provides structural information of the impaired bone and cartilage, not the pathophysiological information associated with different phenotypes that have been clinically manifested, especially at early stage of arthritic development. Therefore, non-invasive methods for defining and identifying sub-classes of OA would be of great significance for personalized treatment, clinical evaluation and prognosis of OA.

Biological fluids, such as urine and plasma, contain a large number of metabolites that can provide valuable bioinformation on organism metabolism (Nicholson et al. 1999; Atherton et al. 2008). Metabolites in biological fluids are in dynamic equilibrium with those inside cells and tissues, the abnormal cellular processes in tissues of an organism following a pathological intervention or disease process can be finally reflected in altered composition of biological fluids (Dunn and Ellis 2005; Griffin et al. 2001; Santini et al. 2004). Recent technological advances in chemical analysis and its combination with sophisticated mathematical models have enabled the application of global metabolic profiling to biological fluids, ultimately leading to identification of candidate biomarkers and altered biochemical pathways, and aiding interpretation of the biological changes caused by diseases (Ellis et al. 2007). Nuclear magnetic resonance (NMR) or mass spectrometry (MS) based metabonomics/metabolomic technology has been effectively applied for evaluation or diagnosis of cancer (Denkert et al. 2006; Beger et al. 2006), coronary heart disease (Brindle et al. 2002; Dunn et al. 2007), type 2 diabetes mellitus (van Doorn et al. 2007; Yi et al. 2006; Qiu et al. 2008) and mental disorders (Holmes et al. 2006; Rozen et al. 2005). We conceive that the noninvasive approach of urinary metabonomic study would identify characteristic metabolic profiles for diagnosis of OA and provide metabolic information linking to the pathological mechanisms of OA (Weljie et al. 2007).

In the present study, we used gas chromatography–mass spectrometry (GC–MS) in combination with multivariate statistical analysis to analyze global changes in urinary metabolites between patients suffering from OA (with or without knee effusion). The study was intended to explore an alternative approach for the diagnosis and stratification of OA patients.

MATERIALS AND METHODS

Experimental participants

The experiment design for this study involved urine from 37 healthy control individuals and two cohorts of OA patients with (n = 20) or without (n = 17) knee effusion. All of the OA subjects were recruited from outpatients of Yueyang Hospital of Integrated Chinese and Western Medicine affiliated to Shanghai University of Traditional Chinese Medicine (Shanghai, China) and control participants were collected from local communities via public advertisement. This experiment was approved by Research Ethics Committee of Yueyang Hospital and written informed consent was obtained from all participants. More details of participants are provided in Table 1.

Table 1: Demographic description of subjects in the study

	Control	*P* value[a]	Knee OA		
			Total	OA1	OA2
Total number	37		37	17	20
Female number	26		30	12	18
Age (years)	56.3 ± 7.9	0.7708	56.8 ± 7.2	55.8 ± 8.7	57.7 ± 5.8
Height (cm)	163.9 ± 6.0	0.3232	162.5 ± 5.9	164.2 ± 6.9	161.2 ± 4.5
Body weight (Kg)	64.1 ± 6.1	0.1997	66.4 ± 9.3	66.7 ± 10.3	66.2 ± 8.6
BMI	23.8 ± 1.9	0.0377	25.1 ± 3.2	24.7 ± 3.5	25.5 ± 2.9
X-ray grade	0	<0.001	1.8 ± 0.6	1.8 ± 0.6	1.9 ± 0.6
Current NSAID use	None				
COX-2 inhibitor use	None				

[a] *P* value: The unpaired *t*-test was conducted to compare the significance of age, height, body weight and BMI between OA patients and healthy controls

Inclusive and exclusive criteria of control individuals

The 37 controls were selected from the healthy population which age, gender matched with that of OA patients. The healthy individuals without any lower limb pathology or joint disorder (injury, trauma, surgery) in either knee in the past year. Participants displayed abnormality on physical examination of the knee (flexion range of motion <125°, knee effusion, ligamentous laxity, meniscus tear) were excluded. Other exclusion criteria included: blood dyscrasias, malignancy, osteoporosis, chondrocalcinosis, thyropathy, tuberculosis, rheumatism or autoimmune disease (psoriasis, lupus) and other inflammatory conditions, corticosteroid injection, or nonsteroidal anti-inflammatory drugs (NSAIDs) use in the past month.

Inclusive and exclusive criteria of OA patients

Diagnosis of knee OA was based on the American College of Rheumatology (ACR) criteria for OA (Altman et al. 1986). A total of 37 OA patients (age range from 45 to 75 years old) have knee pain, aching, or morning stiffness on most days within the last month. Eligible patients were required to display radiological evidence (skyline, weight-bearing Antero-posterior and lateral views) of knee OA on a radiograph obtained within 6 months of the study. All the study candidates had to fill out a questionnaire that screened for other forms of arthritis (for example, rheumatoid arthritis), and information on the use of medications for arthritis was gathered. If a patient screened positive for another form of arthritis or had been receiving medications that were appropriate for rheumatoid arthritis or other forms of arthritis, he or she was excluded. Exclusion criteria also included: knee surgery (previous 12 months), past history of lower limb joint replacement, intra-articular steroid injection (previous 6 months), chondrocalcinosis, blood dyscrasias, malignancy, osteoporosis, medical treatment in the form of acetaminophen, NSAIDs, or selective cyclooxygenase-2 (COX-2) inhibitors in the past 2 months, as well as their classification as radiological grade 4 on the Kellgren–Lawrence (KL) scale (Kellgren and Lawrence 1957) for the study knee or functional knee disability. In patients in whom both knees were symptomatic, we chose the most symptomatic knee for the investigation.

X-rays grade of knee OA

Participants with OA had X-rays (skyline, weight-bearing Antero-posterior and lateral skyline views) of the tested knee. Severity of OA was evaluated according to the KL grading scale: grade 0 for normal, grade 1 for possible osteophytes only, grade 2 for definite osteophytes and possible joint space narrowing; grade 3 for moderate osteophytes and/or definite joint space narrowing; grade 4 for large osteophytes, severe joint space narrowing and/or bony sclerosis

(Kellgren and Lawrence 1957). In this study, 27% of OA patients graded as mild (grade 1, 10/37), 65% graded as moderate (grade 2, 24/37), and 8% graded as severe (grade 3, 3/37).

Phenotypes of OA1 and OA2

OA patients were further divided into two subgroups according to the patellar ballottement test in knee OA: OA1 subgroup (n = 17), in which the patellar ballottement test is negative, and OA2 subgroup (n = 20), in which a positive value for the patellar ballottement test indicates knee effusion. Compared with those of the OA1 group, typical symptoms of OA2 patients usually included concomitant synovitis, the presence of knee effusion and physical signs of inflammation such as swelling and redness. Knee OA magnetic resonance imaging (MRI) was also performed using a 1.0T Gyroscan ACS-NT whole-body scanner (Philips Medical Systems, Best, Netherlands). The knee effusion severity of both OA phenotypes was assessed by MRI.

Patellar ballottement test

Physician put right hand superior to the patella, push the tissues inferiorly towards the patella, then keep this hand in this position holding pressure on these tissues, left hand rapidly press down on the patella and release. A “tapping” (ballottement) of the patella indicate effusion within the knee joint, which is the positive test result. While no “tapping” symptom indicates negative test result.

Urine sample collection and preparation

Second morning voided urine specimens were obtained from OA patients and normal subjects before breakfast, Specimens were aliquoted and then stored at −80°C until GC–MS analysis.

The urine samples for GC–MS analysis were processed as in our previously published two-step ethyl chlorformate (ECF) derivatization method (Qiu et al. 2007) with minor modification. Each 600 µl aliquot of urine sample was mixed with 100 µl L-2-chlorophenylalanine (0.1 mg/ml, an internal standard), 400 µl anhydrous ethanol, 100 µl pyridine, and 50 µl ECF at room temperature for the first step derivatization. The mixture was sonicated at 40 KHz for 1 min to accelerate the derivatization reaction. After adding addition of 300 µl chloroform, the pH of the system was adjusted to 9–10 with 100 µl NaOH (7 mol/l). The second step derivatization was carried out by addition of an additional 50 µL ECF into the above system. The resulting mixture was centrifuged at 3000 rpm for 3 min and the chloroform layer containing derivatives was obtained. Anhydrous sodium sulfate was added to the chloroform layer to remove traces of water before GC–MS analysis.

GC–MS analysis of urine samples

One µL aliquot of the analyte was injected into a DB-5MS capillary column coated with 5% diphenyl cross-linked 95% dimethylpolysiloxane (30 m × 250 µm i.d., 0.25-µm film thickness; Agilent J&W Scientific, Folsom, CA, USA) with a splitless model and analyzed on a combined PerkinElmer gas chromatograph and TurboMass-Autosystem XL mass spectrometer (PerkinElmer Inc., Waltham, MA, USA). The oven temperature was set at 80°C for 2 min, ramped to 140°C at a rate of 10°C/min, to 240°C at a rate of 4°C/min, to 280°C at a rate of 10°C/min, and held finally at 280°C for 3 min. The temperatures of injection, interface, and ion source were set at 260, 260, and 200°C, respectively. Helium was used as the carrier gas at a

flow rate of 1 ml/min. The MS director was operated in the electron energy (EI) model at 70 eV of electron energy with full scan mode (m/z 30–550).

Spectral processing and data analysis

All the unprocessed GC–MS raw files were converted into the NetCDF format via DataBridge (Perkin-Elmer Inc., Waltham, MA, USA) and subsequently processed by the XCMS toolbox (http://metlin.scripps.edu/download) using XCMS default settings. Then, the resulting table (TSV file) was exported into Matlab software 7.0 to perform baseline correction, peak deconvolution and alignment (Ni et al. 2007), finally, the internal standard exclusion and normalization to the total sum of chromatogram were performed in the Excel table resulting from Matlab HDA toolkit. The resulting three-dimensional matrix, including variable indices (RT–*M/Z* pairs), sample names (observations), and normalized peak areas (variables) were introduced into the SIMCA-P 11.0 Software package (Umetrics, Umeå, Sweden) for multivariate data analysis (Jolliffe 1986; London 1987; Bylesjo et al. 2006; Wold et al. 1987). The data were mean-centered and then pareto-scaled. The mean-centering procedure subtracts the mean of the data and results in a shift of the data towards the mean. The pareto-scaling technique gives the weight of each variable by the square root of its standard deviation, which amplifies the contribution of lower concentration metabolites but not to such an extent where noise produces a large contribution, this process facilitates the detection of metabolites consistently present in the biological samples. Initial principal component analysis (PCA) was used to investigate general interrelation including groupings, clustering, and outliers among the observations. To maximize the difference of metabolic profiles between groups, a more sophisticated orthogonal partial least squares projection to latent structure-discriminant analysis (OPLS-DA) model was applied. The OPLS-DA technique is capable of removing information unrelated to the response matrix Y (descriptor, e.g., 0/1) from an input matrix X (GC–MS spectral data), thus the resulting differential metabolites accountable for the discrimination between the two groups are most likely to be concentrated in the first predictive component. The VIP (variable importance in the projection) value of each variable in the model was calculated to indicate its contribution of the **X** variables to the classification. Those variables with a VIP value greater than 1.0 are considered significantly different, and a larger VIP value of a variable represents higher contribution to the discrimination between two groups.

In the SIMCA-P software package, OPLS-DA models were validated by a default 7-round cross-validation procedure with exclusion of 1/7th of the samples from the model in each round in order to avoid the modeling over-fitting caused by supervised mathematical methods. This procedure was repeated in an iterative manner until each sample had been excluded once. The resulting cumulative value of $\mathbf{R^2Y}$ **(**$\mathbf{R^2Ycum}$**)** represents the fraction of the variation of the **Y** variables explained by the model, and provides an estimate of how well the model fits the **Y** data. The cumulative value of $\mathbf{Q^2Y}$ **(**$\mathbf{Q^2Ycum}$**)** obtained from the cross-validation procedure represents the predictive accuracy of the model. $\mathbf{R^2Ycum}$ and $\mathbf{Q^2Ycum}$ values close to 1.0 suggest a satisfactory model with a reliable predictive ability.

The differential metabolites obtained from multivariate data analysis were then validated using Mann–Whitney test at a univariate analysis level (Kruskal and Wallis 1952). The critical *P* value for each test was set at 0.05. The ultimate differentially expressed urinary metabolites were obtained by both VIP value (VIP > 1) and critical *P* values from Mann–Whitney test ($P < 0.05$)

to avoid potentially spurious associations in terms of the borderline number of samples. The unpaired *t*-test was also used to compare the age, height, and body weight between OA patients and normal subjects. Univariate statistical analysis was performed by the statistical toolbox incorporated in MATLAB software (The MathWorks, Inc. Natick, MA, USA).

RESULTS AND DISCUSSION

Clinical characteristics of study population

The characteristics of knee OA patients and the controls are summarized in Table 1. Unpaired *t*-test showed no statistically significant differences in age, height, and body weight between OA patients and normal subjects. These findings indicate that the intra-group differences were mainly due to OA-related pathological variations.

MRI

The sagittal magnetic resonance imaging (MRI) of both knee OA phenotypes was shown in Fig. 1. The image showed that the patellofemoral and tibiofemoral joint of both OA phenotypes appear hyperostosis at different stage. Compared with Fig. 1a, marked effusion existed in suprapatellar bursa of the knee joint, which is the main manifestation of synovitis (Fig. 1b, knee effusion was indicated by arrow).

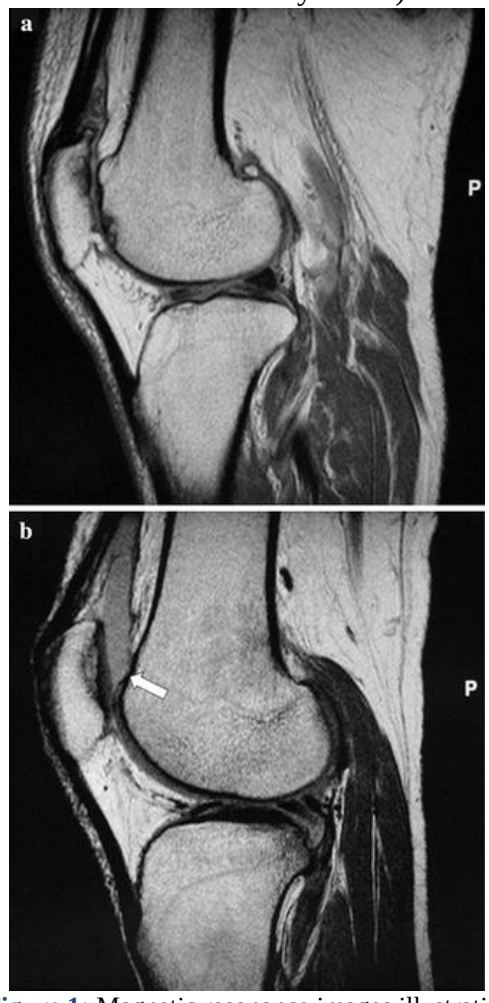

Figure 1: Magnetic resonance images illustrating the severity of OA knee effusion **a** knee joint arthritis without effusion (OA1) and **b** knee joint arthritis with apparent knee effusion in suprapatellar bursa (indicated by *arrow*)

Urinary metabolomic study of OA patients and controls

The typical GC–MS total ion current (TIC) profiles of the Control, OA1 and OA2 urine sample were compared visually (Fig. 2a). Using our optimized GC–MS analysis protocol, we detected about 200–250 different urine metabolites, both healthy control and OA patients, with 65 of them identified using the commercial MS and/or our in-house standard library (the ECF-derivation metabolites library). The metabolites detected in the chromatogram mainly comprised a range of amino acids, organic acids, amines, and phenol-containing compounds (Table 2). These metabolic profiling illustrated the glance of pathophysiological characteristics of control and OA patients. However, it is difficult to be used for OA diagnosis, therefore, multivariate statistical models and bioinformation extraction were performed on the raw GC/MS data files.

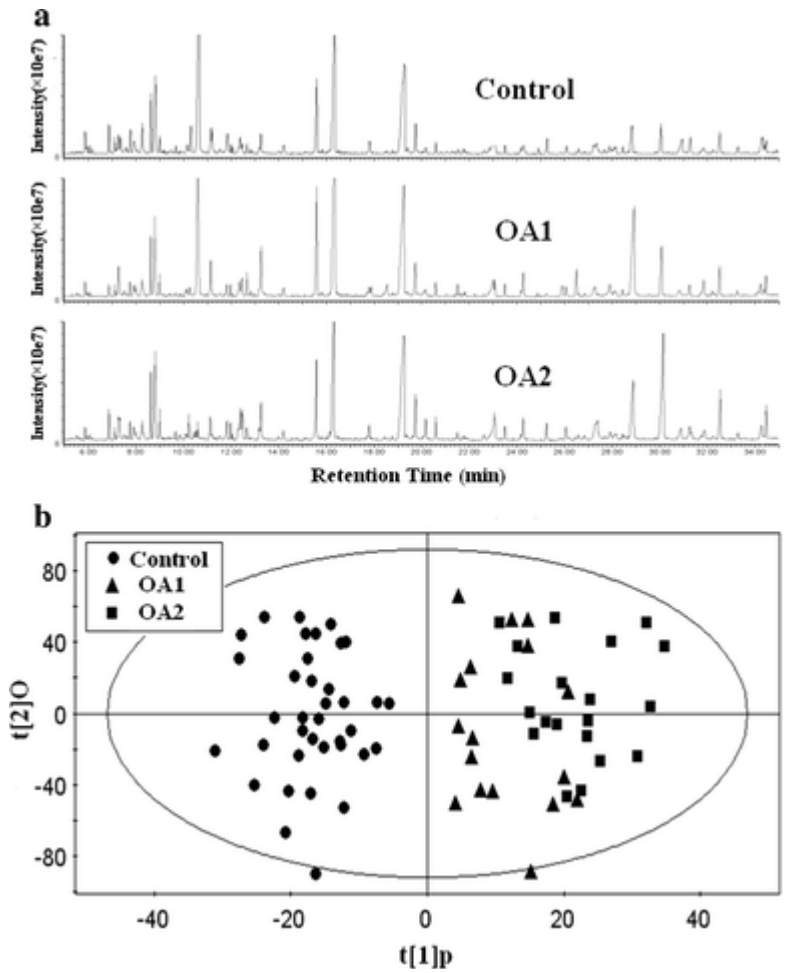

Figure 2: **a** Representative GC–MS spectra of urinary metabolites from normal subjects, OA1 patients and OA2 patients. **b** OPLS-DA scores plot (t[1]P/t[2]O) of GC–MS data derived from the urine samples of normal subjects and OA patients, individuals are illustrated in healthy control group (●), OA1 patients (▲) and OA2 patients (■)

Table 2: Statistical analysis of differentially expressed metabolites (VIP > 1) between OA and control group, and between OA1 and OA2 groups

Key	Rt/min	Metabolites	OA vs. Control				OA2 vs. OA1			
			VIP[a]	Correlation coefficient[b]	*P* (Mann–Whitney)	Fold change[c]	VIP	Correlation coefficient	*P* (Mann–Whitney)	Fold change
1	5.88	4-methyl phenol	2.02	−0.27	0.0059	−1.4	2.17	−0.42	0.0031	−1.7
2	8.79	Glycine	3.18	−0.13	0.0341	−1.3	4.19	0.22	0.0481	1.2
3	15.58	Aconitic acid	7.19	0.27	0.0254	1.3	4.17	0.22	0.0759	1.4
4	15.76	Isocitric acid	1.55	0.15	0.0439	1.2	2.22	0.26	0.0224	1.5
5	15.89	Homovanillic acid	2.04	0.30	0.0225	1.4				
6	16.28	Citric acid	5.21	0.27	0.0311	1.3	4.92	0.32	0.0429	1.4
7	19.18	Hippuric acid	2.61	−0.29	0.0489	−1.2	7.13	−0.34	0.0454	−1.4
8	21.81	Acetoacetic acid	1.94	0.48	0.0007	1.7				
9	22.99	Glutamine	2.16	−0.25	0.0367	−1.3	1.88	−0.31	0.0412	−1.3
10	23.07	4-Methyleneproline	2.42	0.47	0.0004	1.7				
11	27.25	Histidine	1.91	−0.29	0.0162	−1.4	1.90	−0.38	0.0356	−1.4
12	30.00	Histamine	2.21	0.21	0.0224	1.3	1.95	0.36	0.0362	1.4
13	30.83	N-phenylacetyl glutamine					2.49	−0.48	0.0088	−1.6
14	31.28	4-hydroxy hippurate	2.8	0.41	0.0006	1.6	2.27	−0.39	0.0397	−1.5
15	34.24	Tryptophan					2.69	−0.50	0.0014	−1.8

[a]VIP :Variable importance in the projection. [b]Correlation coefficient: Correlation coefficient of obtained from OPLS-DA model, the positive value of correlation means a higher level of metabolites in the former group as compared to the latter group (e.g. OA as former and Control as latter group); whereas the negative value of correlation coefficients represents a lower level of metabolits. [c] fold change: fold change value for specific metabolites was calculated by a non-parametric Mann–Whitney test

To determine whether it was possible to distinguish control and OA patients on the basis of GC–MS chromatogram, PCA and OPLS-DA were carried out in this study. An OPLS-DA model (one predictive component and three orthogonal components) with **R2Ycum** of 0.85 and **Q2Ycum** of 0.50 was achieved. The OPLS-DA scores plot showed that evident clustering of controls and OA patients, and the separation between these two cohorts is significant in the first principle component, which reflected the pathological variation between the OA patients and the normal controls (Fig. 2b). The most important metabolites responsible for the apparent discrimination (those with VIP > 1) are listed in Table 2. The significantly elevated levels of aconitate, isocitrate, citrate, and histamine and the reduced levels of histidine and glutamine were identified in the OA patients compared with the normal controls.

Prediction of OA as potential diagnosis model

Approximately 70% of all the participants (54/74, used as the “training set”) were selected randomly to construct an OPLS-DA model that could be used to predict the class membership of the remaining 30% of the participants (20/74, used as the “test set”). The T-prediction scatter plot with sample number as X-axis and tPS (the first score of T-prediction) as Y-axis was shown as Fig. 3. The star denotes the test samples while the diamond and dot denotes OA and control samples, respectively. It assigned samples to control class and OA class using a cut-off of 0. A total of 17 samples (about 85%) from test set are classified correctly. This procedure (random sample selection, modeling, and prediction) was conducted over 20 times and the results are consistent (the average correct rate is 85%).

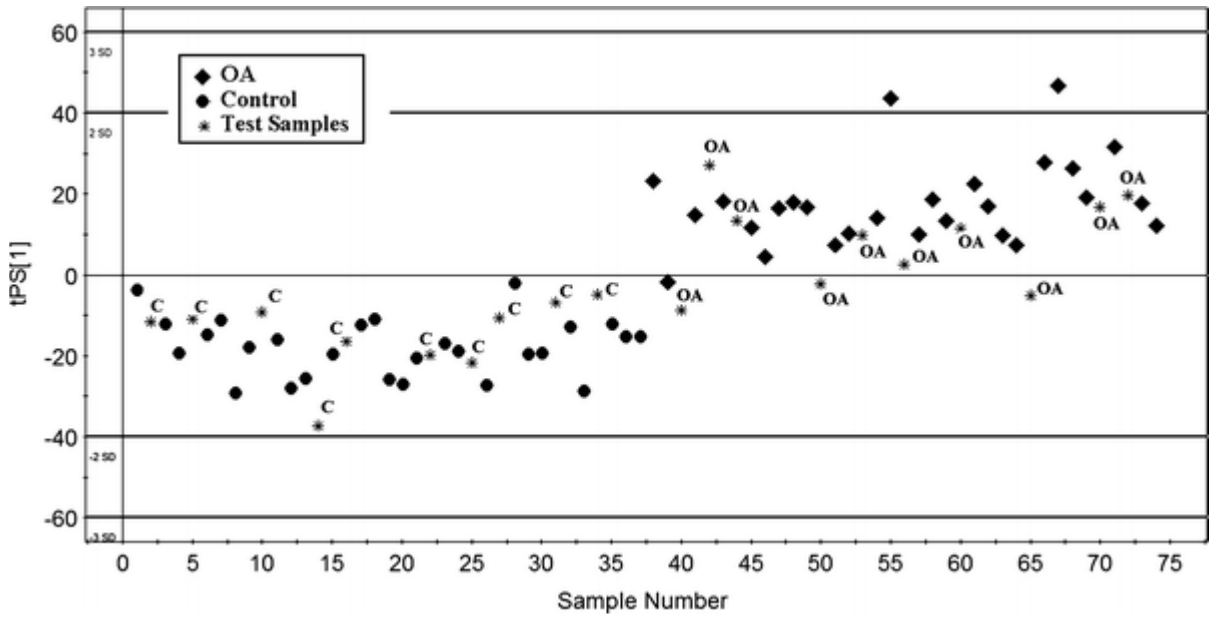

Figure 3: The OPLS-DA prediction model of OA status. An OPLS-DA model was constructed using data from 27 controls (●) and 27 OA patients (◆) (the "training set"), this model was then used to predict OA status of a further 10 samples of each class that were not used in the construction of the model ("the test set"), the test sets were shown as star with their class name (*C denote control "test", *OA denote OA test)

The stratification of OA patients

Further, to determine whether GC–MS based metabolic profiling analysis could distinguish different OA phenotype (OA1 and OA2), the GC–MS raw data from both phenotypes were analyzed by OPLS-DA once again. The OPLS-DA model (one predictive component and two orthogonal components) was established on the metabolic profiles of OA1 and OA2 patients with **R2Ycum** of 0.87 and **Q2Ycum** of 0.70, which reflected the significant separation between these two subsets of knee OA (Fig. 4). The relevant differential expressed metabolites between the two OA phenotypes are also summarized in Table 2. Because histamine and histidine are inflammation related metabolites, the altered expression between OA1 group and OA2 group of these two metabolites are calculated and illustrated in Fig. 5. The level of histamine was higher and level of histidine was lower in OA2 phenotype as compared to OA1 phenotype.

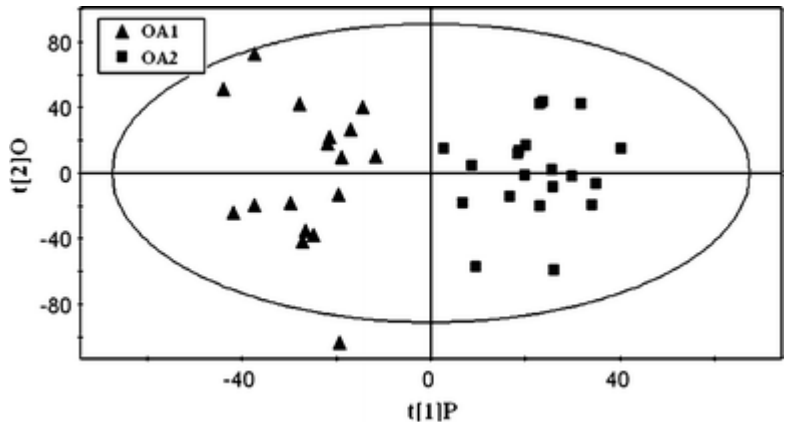

Figure 4: OPLS-DA model comparing the OA1 and OA2 phenotypes. The two subsets are separated in the first principal component. Individuals are illustrated in OA1 patients (▲) and OA2 patients (■)

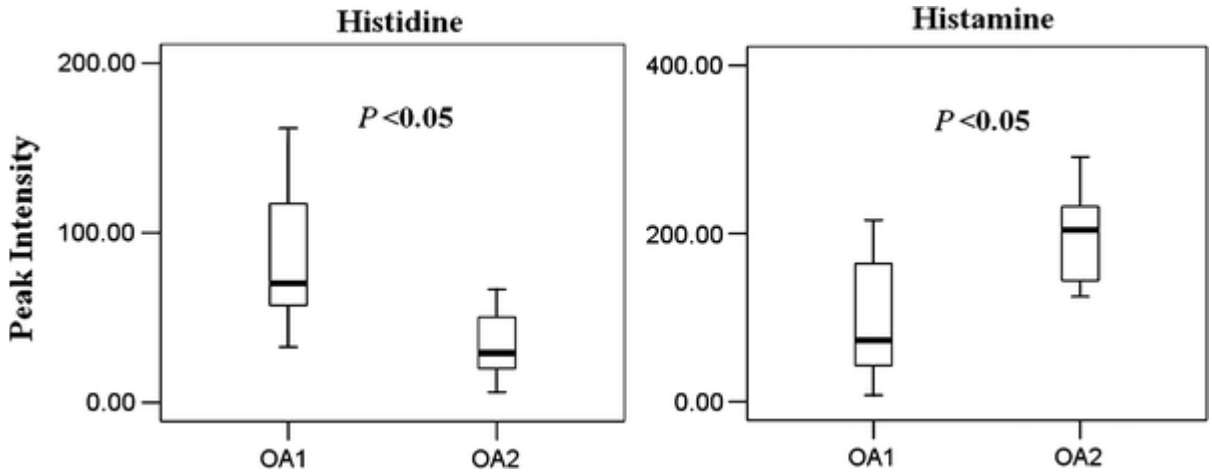

Figure 5: Box and whisker plots demonstrated altered expression of histidine and histamine between OA1 and OA2 phenotypes

The potential biomarkers of OA

NMR-based metabolomics/metabonomics has recently been used to analyze small molecule metabolites from biological fluids to reflect the OA process (Lamers et al. 2005). However, no studies have attempted to further classify the different phenotypes of OA with and without joint effusion. Joint effusion, always accompanied with synovitis which is reflected by a painful and swollen joint, is generally believed to be associated with the progress of bone structure change in OA (Nuki 1999). Comprehensive understanding of metabolic variations associated with OA and joint effusion would be helpful in retarding bone degradation and then finding the OA biomarkers.

Urinary metabolomic study of knee OA patients enhances our understanding of significant metabolic variations associated with OA. For example, elevated expression of aconitic acid, isocitric acid, and citric acid was found in the urine of OA patients, which indicating enhanced activity of the tricarboxylic acid cycle (TCA) as a result of perturbed metabolism in cartilage and chondrocytes. As most of the enzymes involved in the TCA cycle, including citrate synthase (CS), are located in cartilage cell mitochondria, abnormal excretion of TCA substances in the urine of OA patients may provide metabolic evidence for mitochondrial dysfunction in osteoarthritis (Blanco et al. 2004). Glutamine, a major source of energy for connective tissue cells, is required by cartilage cells as an amino donor for the synthesis of cartilage collagen (Handley et al. 1980). The down-regulated excretion of glutamine in OA patients may be an indication of impaired energy metabolism in the cartilage cells. The increased concentration of urinary acetoacetic acid, as part of a regulatory mechanism affecting glucose and lipid metabolism to maintain normal or elevated glycolytic intermediates for the purpose of biosynthesis (Ardawi and Newsholme 1984), suggested that lipolysis may play an important role as energy source in the arthritic knee joint, which was consistent with the previous literature report (Damyanovich et al. 1999).

Significant metabolic differences were also observed between OA1 and OA2 phenotypes, including lower level of histidine and higher level of histamine in OA2 patients as compared to OA1. Histamine, an important modulator of numerous physiological processes including inflammation (Akdis and Blaser 2003), contributes to the articular cartilage degenerative or

catabolic behavior in vitro by stimulating the proliferation of OA human articular chondrocytes (HAC) (Tetlow and Woolley 2003). Mast cells (MCs) are recognized to be a major source of histamine in connective tissues (Dvorak 1998), and increased numbers of MCs and raised levels of histamine have been found in the synovial membrane and fluid, pannus, and bone erosions of OA patients (Malone et al. 1986). In addition, the synthesis of histamine from histidine is catalyzed by histidine decarboxylase (HDC), an enzyme characteristically expressed by MCs, and both increased HDC and histamine were demonstrated in HAC of OA cartilage (Tetlow and Woolley 2005). Therefore, it is likely that the increase in urinary histamine is a result of increased HDC activity in OA, resulting in decreased histidine concentration in OA2 patients.

Routine laboratory tests used for OA, such as erythrocyte sedimentation rate (ESR) and serum C-reactive protein level, are not specific for OA patients with apparent symptoms of inflammation such as knee effusion, and these inflammatory changes are evident relatively late in the pathological progression of knee OA. In the present study, the use of X-ray radiographic result could not provide sufficient information for classification of the two different phenotypes of OA patients (Table 1; X-ray grade, OA1 1.8 ± 0.6 vs. OA2 1.9 ± 0.6). Alternatively, GC–MS based metabolic profiling with supervised OPLS-DA technique could be used as a novel approach in differentiating the two OA phenotypes.

CONCLUDING REMARKS

In this metabolomic study, a noninvasive GC–MS based urinary metabolite analysis combined with a multivariate statistical technique is able to detect metabolic profile variations between OA patients and healthy controls, as well as between two different OA phenotypes. The OPLS-DA and T-prediction model demonstrated good classification of disease group from healthy subjects, indicating its great potential of diagnostic application in OA. In addition, further analysis of differential metabolites revealed an up-regulated TCA cycle associated with OA, and histidine metabolism disorders accompanied with knee effusion symptoms, which could be used as potential biomarkers for OA.

ABBREVIATIONS

BMI	Body mass index
COX-2	Cyclooxygenase 2
CS	Citrate synthase
GC–MS	Gas chromatography–mass spectrometry
HAC	Human articular chondrocytes
HDC	Histidine decarboxylase
KL	Kellgren–Lawrence
MCs	Mast cells
MRI	Magnetic resonance imaging
NMR	Nuclear magnetic resonance
NSAIDs	Nonsteroidal anti-inflammatory drugs
OA	Osteoarthritis
OPLS-DA	Orthogonal partial least squares projection to latent structure-discriminant analysis
PCA	Principle component analysis
TCA	Tricarboxylic acid

TIC	Total ion current
VIP	Variable importance in the projection

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