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Exploration of candidate biomarkers for human psoriasis based on gas chromatography-mass spectrometry serum metabolomics

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Summary

Background Recent studies have shown that dysregulated metabolic pathways are linked to psoriasis pathogenesis. However, an extensive, unbiased metabolic analysis in patients with psoriasis has not been completely explored. The metabolome represents the end products of proteomics or cellular processes that may be closely associated with the pathogenesis of psoriasis.

Objectives To determine the differences in serum metabolomic profiles among patients with psoriasis and healthy controls with the goal of identifying potential biomarkers in patients with psoriasis.

Materials and methods Serum metabolomic profiles from 29 subjects (14 patients with psoriasis and 15 sex- and age-matched healthy controls). The serum metabolites were analysed by gas chromatography-mass spectrometry based on a combined full scan and selected-ion monitoring mode.

Results Multivariate statistical analysis of metabolomics data revealed altered serum metabolites between the patients with psoriasis and healthy individuals. Compared with healthy individuals, patients with psoriasis had higher levels of amino acids including asparagine, aspartic acid, isoleucine, phenylalanine, ornithine and proline; higher levels of lactic acid and urea; and lower levels of crotonic acid, azelaic acid, ethanolamine and cholesterol.

Conclusions It appears that the glycolysis pathway and amino acid metabolic activity are increased in patients with psoriasis. These metabolic perturbations may stem from increased demand for protein biosynthesis and keratinocyte hyperproliferation. Our findings may help to elucidate the pathogenesis of psoriasis and provide insights into early diagnosis and therapeutic intervention.

What's already known about this topic?

- Three significant metabolic pathways have been found to be associated with psoriasis disease severity: arginine and proline; glycine, serine and threonine; and alanine, aspartate and glutamate.
- Compared with controls, patients with psoriasis have a higher level of alpha ketoglutaric acid but a lower level of asparagine and glutamine.

What does this study add?

- Patients with psoriasis showed higher levels of amino acids; lactic acid and urea; add lower levels of crotonic acid, azelaic acid, ethanolamine and cholesterol, compared with healthy controls.
- Our study shows that ornithine, hypoxanthine, crotonic acid and azelaic acid might be the most significant biomarkers for patients with psoriasis.

What is the translational message?

- The metabolites related to glycolysis, protein biosynthesis, redox balance and anabolic activity may be used either for early diagnosis or as a prognosticator for 'health', while having a genetic background of psoriasis without clinical manifestation.

Psoriasis is one of the most common chronic and immune-mediated skin disorders associated with remarkable morbidity and mortality.[1] There are several types of psoriasis. The common features are skin thickening, erythema and pustular or squamous plaque formation, and they also have various complications.[2–7] A complex interaction between genetic and environmental as well as immunological factors initiates a cascade of events that lead to activation of dendritic cells.[8] It involves triggering the immune system, generates a sustained inflammatory reaction and dysregulation of keratinocyte differentiation.[9] Previous studies have shown that T-helper (Th)1 and Th17 effector T cells as well as innate dermal γδ T cells promote the recruitment of inflammatory cells to the skin, stimulate keratinocyte proliferation and sustain a state of chronic inflammation through cytokine production.[10] However, the exact cause of psoriasis remains unclear and need further investigation.

Metabolomics, the most recent addition to the 'omics' fields, is a constantly growing field in systems biology. Its important role among other 'omics' depends on the capacity to identify alterations in all low-molecular mass metabolites in the metabolome of an organism occurring at the cellular level.[11] These small-molecule metabolites indicate the results of sophisticated interactions between genetic inheritance and various environmental stimuli. Genomics, proteomics and transcriptomics research have contributed extensively to the increasing body of investigation regarding psoriasis pathogenesis.[12–15] Thus, examining the metabolic byproducts produced at the transcriptional and translational levels may provide clues to the cellular regulatory processes and underlying molecular networks. Because metabolites are the end products and the most downstream representation of cellular metabolism, comprehensive metabolomics analysis will enable us to gain valuable information about the physiology of a biosystem by detection of amplified end products that result from genetic and environmental perturbations.[16] A combined metabolomics approach [full-scan and selected-ion monitoring (SIM) mode] was applied in this study, using gas chromatography-mass spectrometry (GC-MS) to compare the serum metabolic profiles of patients with psoriasis and healthy controls.

An untargeted metabolomics strategy employing the full-scan mode[17–19] and a targeted approach utilizing the SIM mode[20] of the GC-MS analytical platform are the two main strategies in biomarker discovery. The untargeted method can acquire unbiased full-scan information of metabolites to simplify the qualitative analysis of metabolites, while also being able to detect as many metabolites as possible in one analysis run.[21,22] However, the full-scan method has low sensitivity because of its wide mass scan range and difficulty in peak alignment, in addition to the low resolution of overlapping chromatographic peaks. In contrast, the targeted SIM-mode method is primarily focused on the quantitative analysis of the quantified metabolites. A combined analytical method,[23] which integrates the advantages of unbiased measurements for metabolites using the full-scan mode and the high sensitivity of the SIM mode, has been developed to improve detection sensitivity, data quality and reproducibility, and wide linearity range in our discovery of psoriasis serum biomarkers. The integrated metabolomics approach based on GC-MS was applied to characterize the metabolic profiles of the patients with psoriasis in this study, with the aid of univariate and multivariate statistical analyses, as well as metabolic pathway analysis. The findings from those studies provide new insights into the aetiopathogenesis of psoriasis.

Materials and methods

Chemicals

Methanol (HPLC grade) and dichloromethane were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). MSTFA (N-methyl-N-(trimethylsilyl)-trifluoroacetamide), TMCS (chlorotrimethylsilane), pyridine (HPLC grade), methoxyamine hydrochloride and tridecanoic acid (used as internal standard) were all obtained from Sigma-Aldrich (St Louis, MO, U.S.A.). The standard chemicals for structural identification were obtained from Sigma-Aldrich and the Aladdin Industrial Corporation (Shanghai, China).

Study design

This prospective study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University, Shanghai. Informed consent was obtained from all participants. Healthy controls as well as patients with stable psoriasis vulgaris were recruited at Ruijin Hospital. None of the controls had diabetes, hypertension, hyperlipidaemia or other obesity-related metabolic diseases. None of the patients were prescribed anti-inflammatory drugs and treatment for about 4 weeks. For serum collection, random whole blood was collected, and

then left standing for 1 h at room temperature before centrifugation for 10 min at 3000 rpm. Serum samples were aliquoted and immediately stored at −80 °C until use.

Sample preparation

After thawing at room temperature for 15 min, 50 μL of serum from each sample was pipetted into Eppendorf tubes and equal aliquots of all analytical samples were drawn and mixed completely for 5 min for the preparation of full-scan samples. Each full-scan sample was treated in the same way as other analytical samples. Next, 200 μL of methanol (including 40 μg mL^{-1} tridecanoic acid) was added to 50 μL of serum sample and vortex-mixed for 5 min to precipitate proteins as completely as possible and then left standing at room temperature for 10 min. After centrifugation at 12 000 rpm for 15 min at 4 °C, 150 μL supernatant was collected and transferred for drying under a nitrogen stream at 40 °C. Thereafter, 50 μL of methoxyamine pyridine solution (15 mg mL^{-1}) was added to the dried sample, and vortex-mixed for 5 min to dissolve as many metabolites as possible. Then, a 2-h oximation reaction was conducted in a 40 °C oven, followed by silylation reaction with 40 μL of MSTFA (containing 1% TMCS) in a 40 °C oven for 1 h. After the derivatization reaction, the solution was centrifuged at 12 000 rpm for 5 min. Finally, the supernatant of the derivatized solution was transferred to a 2-mL glass vial with an inserted liner and analysed by GC-MS afterwards.

Data acquisition of the full-scan sample

Serum data acquisition of the full-scan sample was analysed using the GC-MS analytical system with a 7890B GC and 5977A quadrupole mass analyser (Agilent Technologies, Santa Clara, CA, U.S.A.). Chromatographic separations of metabolites were accomplished on a HP-5 MS capillary column (30 m × 250 μm × 0·25 μm). The injection volume and split ratio were set to 1 μL and 2 : 1, respectively. The flow rate of helium was operated at 1·2 mL min^{-1} and temperatures of inlet, interface and ion source were controlled at 300 °C, 280 °C and 230 °C, respectively. The oven temperature was initially set to 70 °C, kept for 3 min, and then increased to 300 °C at a rate of 5 °C min^{-1}, and finally held for 5 min. The gain factor was set at 2·0, and electron impact (70 eV) was applied as the ionization mode. The mass signal acquisition (33–600, m/z) was performed after 6·0 min of solvent delay.

Deconvolution and identification of full-scan data

Raw GC-MS data were first imported into AMDIS 32 version 2·70 and MassHunter Qualitative Analysis for mass spectra detection, deconvolution and identification. The signal-to-noise ratio was set to 20, the absolute peak height and area were set to 2000 and 10 000, respectively, in the process of deconvolution mass spectra using MassHunter. Parameters for mass spectra detection and deconvolution using AMDIS were set with the default values. The metabolites were identified through a mass spectra search against those standards in a commercial library (NIST 11) and the available reference standards.

Establishment of selected-ion monitoring table

The establishment of a SIM table was carried out according to the mass spectrum detection and deconvolution in the full-scan sample, obtaining the time of starting, vertex and terminal points of the metabolite peaks, feature-ion selection and grouping. In order to improve the MS responses of lower-content metabolites, the split ratio in full-scan mode was reduced relative to the SIM mode.

Data acquisition in the selected-ion monitoring mode

For the acquisition of metabolic profiling data in the SIM mode, all samples were analysed using the integrated SIM table; the injection volume and split ratio were set to 1 μL and 20:1, respectively. Other GC-MS parameters were the same as those previously described in the full-scan mode. A cocktail of four external standards were employed as the quality control (QC) samples, then treated and analysed in the same way as other samples to monitor the reproducibility and repeatability of the instrument and method. The serum samples were randomly coded and processed alternately on the basis of the sample categories, followed by six injections of the same QC sample to balance the analytical system and the column. Two QC samples were inserted every 10 analytical samples to supervise the reproducibility and stability of the method. On the other hand, a dichloromethane solution was run every 10 samples (QC samples excluded) as well, for the elution of residual metabolites primarily from the inject inlet, transfer liner and the capillary column. After running all serum samples, the QC sample was injected six times to balance the analytical system and evaluate the reproducibility and repeatability of the analytical sequence.

Data processing

A quantitative table of the unique ions for detected metabolites was created according to the final SIM table and imported into MassHunter Qualitative Analysis for batch integration of the analytical samples. Afterwards, the ion peak area of the metabolite was normalized by dividing by the internal standard peak and multiplying by 1×10^6 using Microsoft Excel and then loaded to SIMCA-P (Umetrics), which was transformed by autoscaling for further data processing. Statistical models including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were established to represent the major latent metabolites in the data matrix. Furthermore, 200 permutations of cross-test

validation, which had abundant mathematical foundation to evaluate the generalization ability of fitting models, were applied to generate reliable results through random division of samples.

PCA was first utilized to visualize the distribution of control and psoriasis samples for the evaluation of serum metabolic profiling. The discriminating metabolites were obtained from the PLS-DA model where the metabolites with variable importance in the projection (VIP) values higher than 1 were selected. Comparisons were made using the t-test and the P-value cut-off was set to 0·05. A heat map employing MeV 4·9 was carried out to project the metabolic regulations of the remarkable shifts in metabolites. The receiver operating characteristic (ROC) curves of the potential biomarkers were made by using Medcalc software. Pathway analysis was performed by means of MetaboAnalyst 3·0, which was used to test for significant enrichment in KEGG pathways among the remarkable metabolic perturbations.

Table 1 Demographic information on the study cohort

	Control	Psoriasis
Female/male	4/11	4/10
Age (years), mean ± SD	49·0 ± 13·4	51·9 ± 10·8
Duration (years)	NA	22·7 ± 9·8
BMI	NA	23·7 ± 3·2
PASI	NA	11·4 ± 7·6

BMI, body mass index; NA, not available; PASI, psoriasis area and severity index. P-value > 0·05 for t-test for age and BMI between controls and subjects with psoriasis.

Results

Workflow of serum metabolomics profiling

The serum recruitment group consisted of 29 sex- and age-matched individuals. Patient demographic information is summarized in Table 1. We analysed metabolites of the mixed serum sample collected from 29 individuals by full-scan analysis using the GC-MS systems. After data preprocessing, 108 peaks (classified in 42 groups) were defined in the final SIM table. After 29 cases of serum SIM data acquisition, peak identification and refining based on the 80% rule[24], 80 metabolites were detected, 63 metabolites were identified and 36 metabolites were confirmed using reference standards (Table S1). Among all the detected metabolites, 20 identified metabolites were among the 24 potential biomarkers in the MetaboAnalyst library of pathway analysis. The workflow of our GC-MS-based full-scan and SIM strategies for serum biomarker discovery is provided in Figure 1.

Multivariate statistical analysis

Plotted PCA scores showed relative obvious clustering for the two groups with the cumulative R2X 0·41 for this model (Fig. 2a). Similarly, the PLS-DA scores plot exhibited distinct separation between the two groups with the cumulative R2X 0·29, R2Y 0·91 and Q2 0·76 (Fig. 2b). The substantial separation of the control and psoriasis samples indicates the impact of the disease condition on the metabolic profile. The OPLS-DA scores plot displayed clear grouping in the two groups with the cumulative R2X 0·36, R2Y 0·95 and Q2 0·76 (Fig. 2c). Two hundred permutations of cross-test validation of the PLS-DA

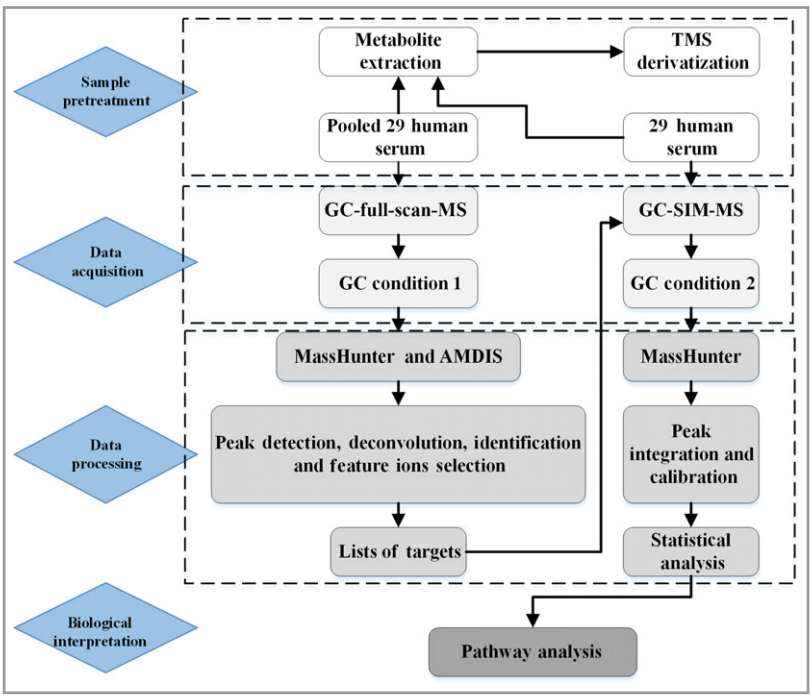

Fig 1. Workflow of our GC-MS based on full-scan and SIM strategies for serum biomarker discovery. GC condition 1: injection volume and split ratio were adjusted to 1 μL and 2 : 1, respectively, which was used specifically to acquire trace metabolite information; GC condition 2: injection volume and split ratio were set to 1 μL and 20 : 1, respectively. GC, gas chromatography; MS, mass spectrometry; SIM, selected-ion monitoring; TMS, trimethylsilyl.

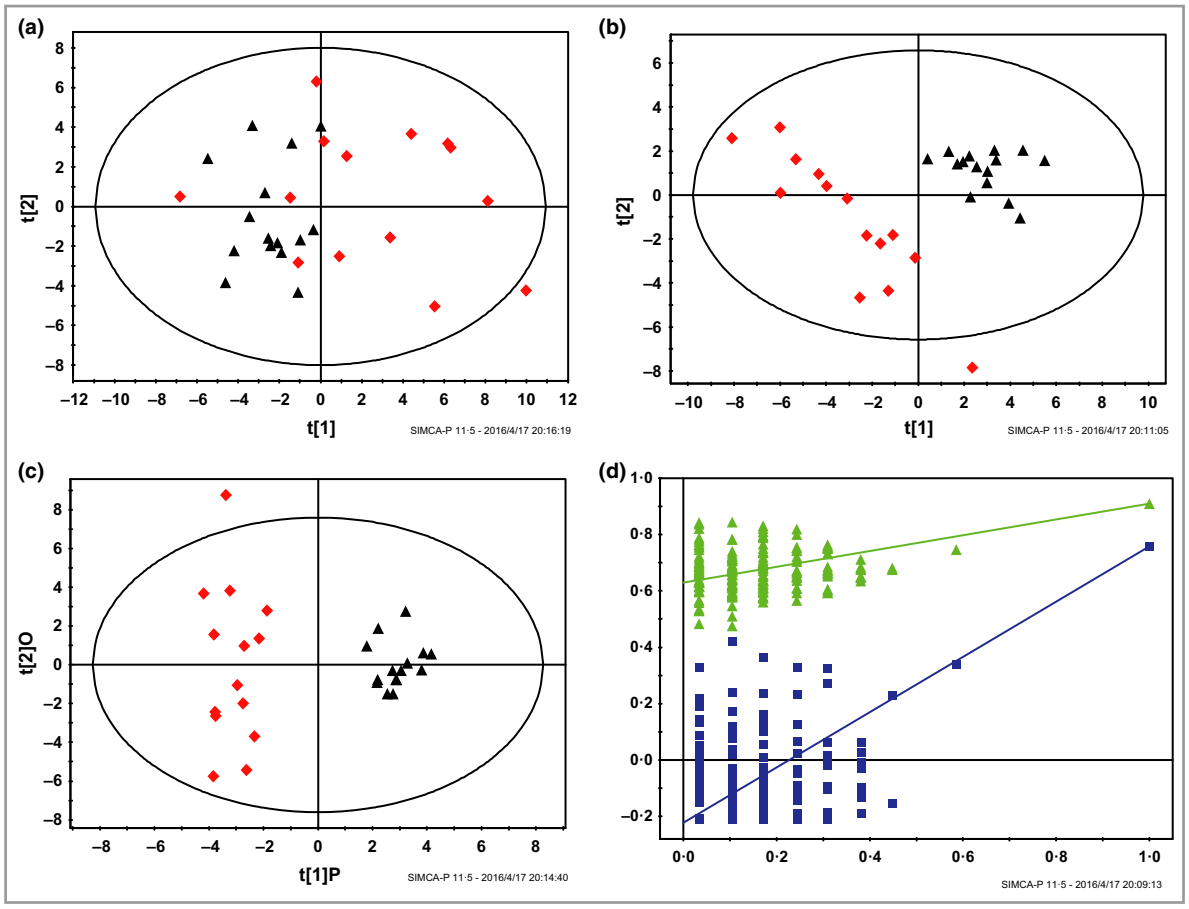

Fig 2. Scores plots obtained by PCA (a), PLS-DA (b) and the OPLS-DA model (c) with healthy controls labelled by black triangles and patients with psoriasis by red boxes. (d) The corresponding validation plot of the PLS-DA model derived from the GC-SIM-MS metabolite of human serum with R2 labelled by green triangles and Q2 labelled by blue boxes. GC-SIM-MS, gas chromatography-mass spectrometry selected-ion monitoring; OPLS-DA, orthogonal partial least squares discriminant analysis; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis.

model showed R2 (0·0, 0·63) and Q2 (0·0, –0·22), indicating satisfactory effectiveness of the model (Fig. 2d).

Screening potential biomarkers

Based on the VIP value of metabolites in the PLS-DA model and t-test discriminating between the control and psoriasis groups, a host of remarkable metabolites (VIP > 1 and $P < 0·05$) were selected as potential biomarkers, most of which are identified and listed in Table 2. Relative levels of the remarkable potential biomarkers in the serum of the two groups are shown in Figure 3. The major metabolic alterations in psoriasis were visualized in a plot (Fig. 4). On the basis of the heat map results of 24 differential metabolites, the distribution of metabolites could be divided visually into upregulated and downregulated. It was observed that a large proportion of amino acids were increased in patients with psoriasis compared with healthy controls. In contrast, fatty acids and derivatives were reduced or not detected in the disease condition (Fig. 4). This suggests metabolic shifts of metabolites in key pathways and the metabolic behaviour of psoriasis, which could be attributed to protein synthesis of biomass for the repair of skin biological systems.

Receiver operating characteristic curve

ROC curve analysis is generally considered to be the gold standard for the assessment of biomarker performance. The results of ROC curve analysis of the 24 differentiated metabolites were on the basis of these outcomes of univariate and multivariate statistical analyses, which should guarantee the reliability of potential biomarkers for wide and qualified independent validation. In addition, high-performance prediction of potential biomarkers was readily achieved, as the area under the curve (AUC) value for each metabolite (except urea and the metabolite with RT = 22·03) was higher than 0·7 (Table S2). Typical ROC curves of AUC values greater than 0·8 are depicted in Figure 5.

Metabolic pathway analysis

A functional pathway analysis facilitating further biological interpretation was subsequently performed to reveal the most relevant pathways as shown in Figure 6.

Table 2 Alterations of the differential metabolites among the samples

Metabolites	Related pathways	VIP	P	FC	p(corr)	Regulation
Lactic acid	Glycolysis and pyruvate metabolism	1·33	0·007	1·71	−0·75	Up
Crotonic acid	Fatty acid biosynthesis	2·53	0·000	0·00	0·83	Down
Unknown (RT = 11·46)	Unknown	1·10	0·020	1·41	−0·46	Up
Valine	Valine, leucine and isoleucine biosynthesis	1·28	0·024	1·25	−0·74	Up
Ethanolamine	Phospholipid biosynthesis	1·01	0·033	0·94	0·45	Down
Urea	Urea cycle	1·00	0·035	1·20	−0·52	Up
Isoleucine	Valine, leucine and isoleucine biosynthesis	1·38	0·005	1·44	−0·78	Up
Proline	Arginine and proline metabolism	1·22	0·039	1·43	−0·71	Up
Erythritol	NA	1·61	0·001	0·72	0·44	Down
Aspartic acid	Alanine, aspartate and glutamate metabolism	1·29	0·005	1·40	−0·67	Up
Unknown (RT = 22·03)	Unknown	1·11	0·042	1·40	−0·64	Up
Glutamic acid	D-glutamine and D-glutamate metabolism	1·11	0·028	1·44	−0·62	Up
Phenylalanine	Phenylalanine metabolism	1·36	0·004	1·26	−0·75	Up
Asparagine	Alanine, aspartate and glutamate metabolism	1·29	0·011	1·34	−0·74	Up
Unknown (RT = 24·22)	Unknown	2·00	0·000	83·45	−0·85	Up
2-Hydroxysebacic acid	Fatty acid metabolism	1·61	0·000	0·48	0·82	Down
Azelaic acid	Fatty acid metabolism	2·38	0·000	0·00	0·82	Down
Hypoxanthine	Purine metabolism	1·62	0·002	6·10	−0·38	Up
Ornithine	Urea cycle	1·47	0·004	1·55	−0·84	Up
13-Octadecenoic acid	Fatty acid metabolism	1·27	0·006	0·37	0·62	Down
Unknown (RT = 38·89)	Unknown	1·53	0·001	1·56	−0·61	Up
Inositol phosphate	Myo-inositol biosynthesis	1·17	0·013	2·33	−0·51	Up
Glycerol 1-hexadecanoate	NA	1·16	0·043	1·23	−0·25	Up
Cholesterol	Primary bile acid biosynthesis	1·27	0·007	0·84	0·69	Down

FC, fold change, mean value of peak area obtained from the psoriasis group/mean value of peak area obtained from the control group. If FC value is < 1, it means that metabolites are fewer in patients with psoriasis than in healthy controls. RT, retention time; Unknown, not identified by library and standards; NA, not available; p(corr), Pearson correlation coefficient.

Discussion

Psoriasis is defined as an autoinflammatory disease condition of the skin that is more than 'skin deep'. The infiltration of inflammatory cells in psoriatic skin lesions may be trafficked into systemic circulation, contributing to chronic systemic inflammation.[10] Thus far, there has been limited work illuminating the underlying metabolic processes and pathways associated with the disease.[25] However, distinct changes in serum metabolic profiles of patients with psoriasis were observed. We show that circulating amino acids, i.e. ornithine, proline, asparagine, aspartic acid, glutamic acid and phenylalanine are elevated in the psoriasis group in accordance with published literature,[26] while azelaic acid, crotonic acid, 13-octadecenoic acid, 2-hydroxysebacic acid, erythritol, ethanolamine and cholesterol are downregulated.

There are metabolic signatures that are associated with psoriasis. Branched chain amino acids (BCAAs), i.e. valine, leucine and isoleucine, have been reported to have relations with several types of disease and cancer.[27,28] BCAAs can activate mammalian target of rapamycin (mTOR, a protein kinase responsible for cell proliferation, cell survival and protein synthesis[29]) signalling pathway and boost growth and proliferation of myocytes and epithelial cells, which is why BCAAs are increasingly applied as an effective treatment against tumours. The upregulated BCAAs in our psoriasis serum samples may have a pathogenic effect in the skin. We have found valine and isoleucine in our SIM analysis to be significant (VIP score > 1 and P-value < 0·05).

Psoriasis is characterized by highly efficient cellular keratinocyte proliferation, which increases the demand of amino acids, notably glutamine, to accommodate for the higher rate of protein synthesis.[30] A remarkable increase in lactic acid levels (psoriasis vs. control, P = 0·007) and a decrease in erythritol levels (P = 0·001) in patients with psoriasis indicated that glycolytic activity was largely increased. Enhanced glycolytic activity, as observed in patients with psoriasis, could supply a beneficial microenvironment for keratinocyte proliferation. Protein synthesis demand in the proliferating skin could also explain the upregulated levels of amino acids in serum. Glutamic acid is a critical amino acid for glutathione homeostasis, which plays a very important role in regulating redox states *in vivo* and removing reactive oxygen species (ROS).[31] Under hypoxic conditions, elevated ROS activity can generate oxidative stress, which leads to glutathione oxidization and initiates glutathione resynthesis.[32]

Moreover, psoriasis also has a relationship with an increase in ornithine of the urea cycle. Remarkable differences in arginine and urea cycle metabolism have been reported previously in psoriatic skin lesions,[33] and similar changes in the intermediates of the urea cycle,[34] similar to alterations in glutamic acid and glutamate, have connections with wound healing.[35] The

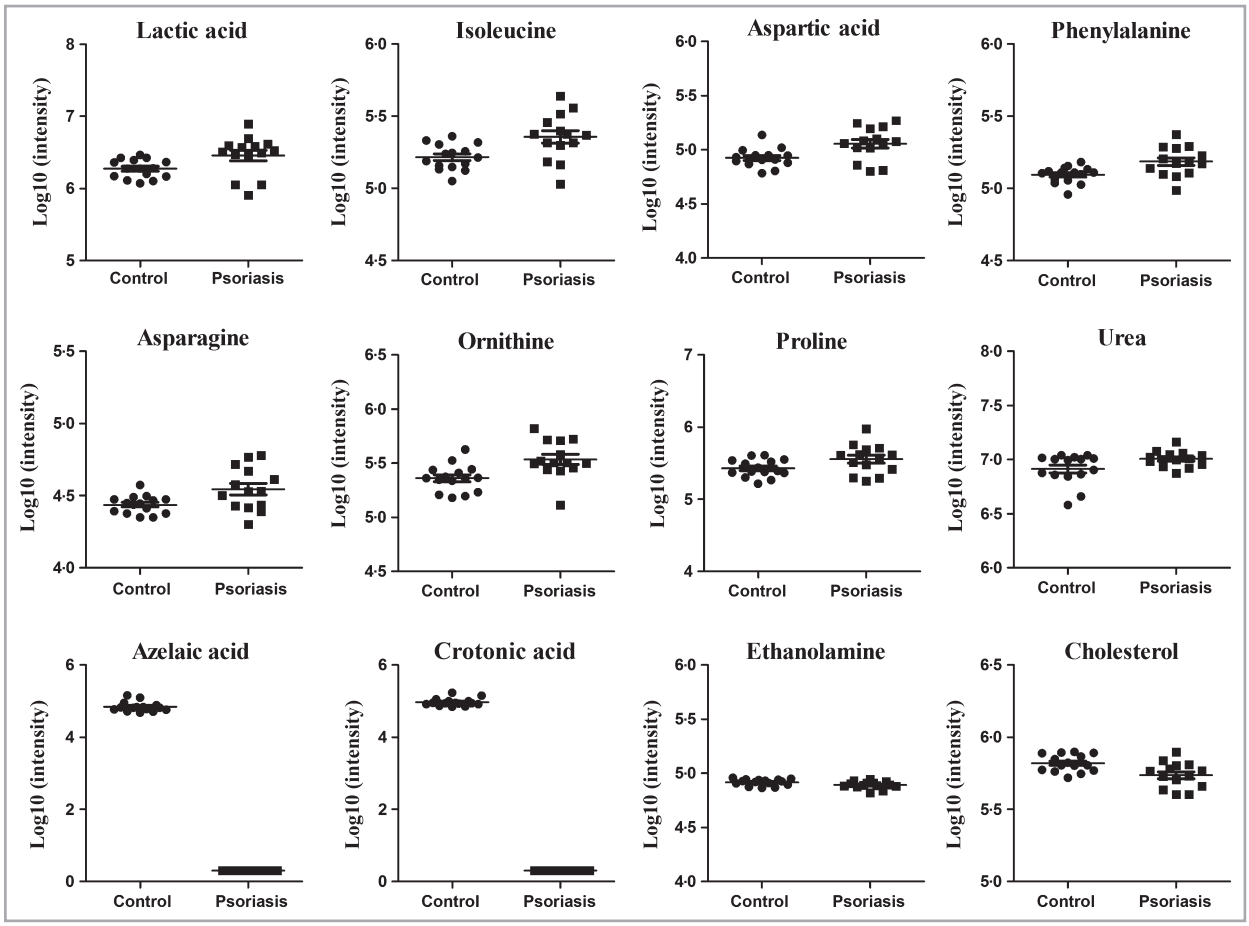

Fig 3. Metabolites with significant alterations in their levels in psoriasis vs. control based on the analysis of serum by GC-SIM-MS. The metabolites in the top two rows show an increasing trend with the progression of psoriasis. The metabolites in the bottom row are downregulated in psoriasis vs. healthy controls. GC-SIM-MS, gas chromatography-mass spectrometry selected-ion monitoring.

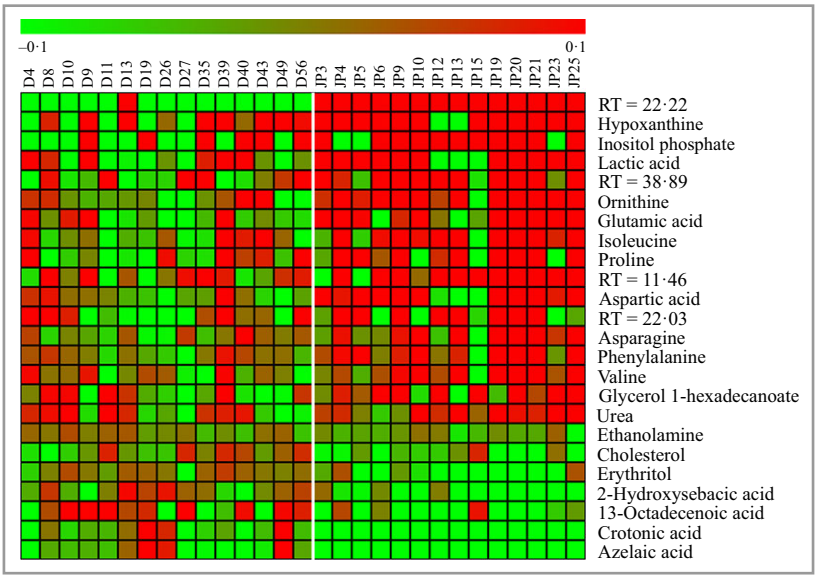

Fig 4. Heat map of the 24 differential metabolites listed in Table 2. The ratio of metabolite in the subject samples to the average of those in the healthy control samples was first calculated, and then the metabolic alteration was demonstrated as log10 (ratio). Healthy controls are labelled D. . . and patients with psoriasis are labelled JP. . .

urea cycle is an entry to the polyamine synthesis pathway and polyamines can stimulate cell proliferation such as keratinocyte expansion, a hallmark of keratinocytosis in psoriasis.[36] The requirement of polyamines may accelerate the mobilization of arginine (the urea cycle intermediate) from synthesis sites to the epidermis, leading to elevated serum levels.[33]

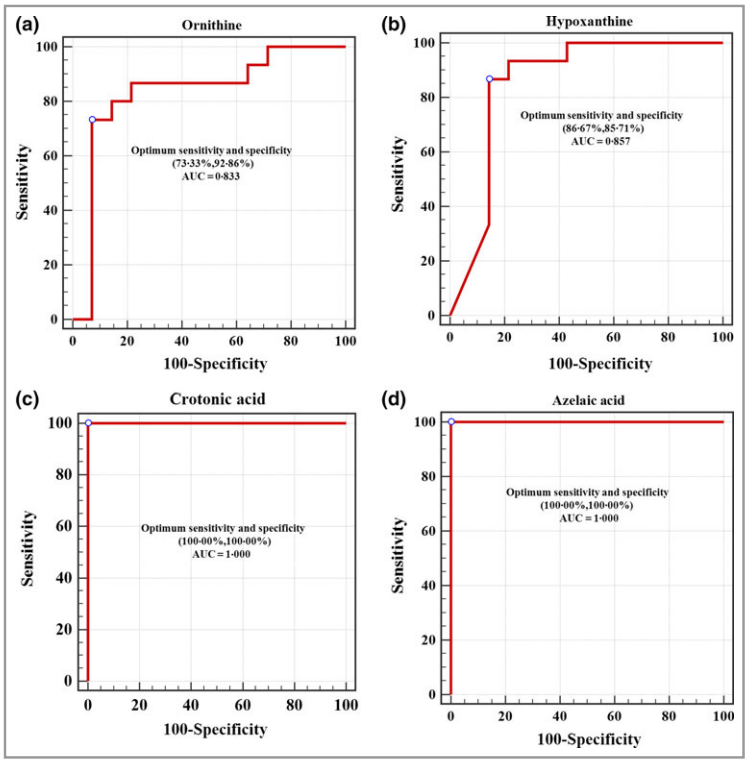

Fig 5. The typical ROC curve plots of potential biomarkers with high-performance prediction, several metabolites of AUC values > 0·8 were exhibited. Ornithine (a), hypoxanthine (b), crotonic acid (c) and azelaic acid (d). AUC, area under the curve.

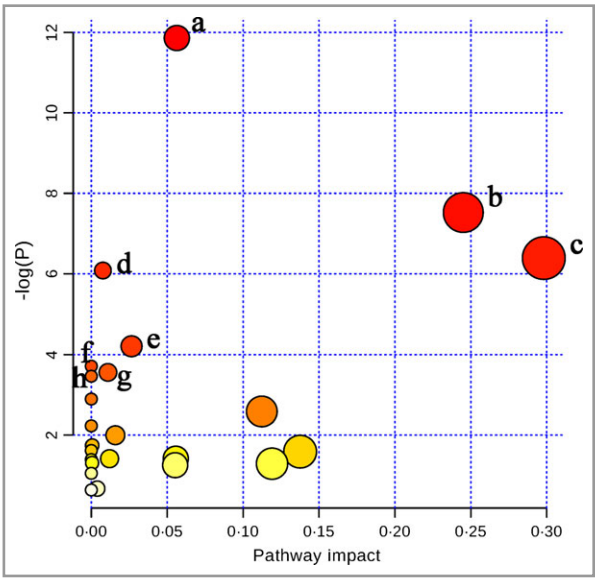

Fig 6. A systemic view of disordered metabolic pathways that associate with the psoriasis group in this study, providing a disease-specific picture of human physiology. The colours (varying from yellow through to red) mean the metabolites are in the data with different levels of significance, red being a more significant pathway than yellow. All metabolic pathways are described according to P-values from the pathway enrichment analysis (*y*-axis) and pathway impact values from the pathway topology analysis (*x*-axis). (a) Aminoacyl-tRNA biosynthesis; (b) alanine, aspartate and glutamate metabolism; (c) arginine and proline metabolism; (d) nitrogen metabolism; (e) valine, leucine and isoleucine biosynthesis; (f) propanoate metabolism; (g) glutathione metabolism; (h) valine, leucine and isoleucine degradation.

The high-performance prediction of crotonic acid and azelaic acid was readily achieved; the AUC value for each was 1·0, which significantly decreased in patients with psoriasis. Crotonic acid and azelaic acid might be the most significant biomarkers associated with the pathogenesis of psoriasis. Crotonic acid is involved in fatty acid biosynthesis and formed by the action of fatty acid synthases from acetyl-CoA and malonyl-CoA precursors. Azelaic acid possesses a variety of biological actions both *in vitro* and *in vivo*. Interest in the biological activity of azelaic acid arose originally out of studies of skin surface lipids and the pathogenesis of hypochromia in pityriasis versicolor infection. Azelaic acid is another option for the topical treatment of mild to moderate inflammatory acne vulgaris.[37,38]

A combined metabolomics approach was applied for delineating the metabolic regulations and discovering potential biomarkers for psoriasis. Psoriasis is clearly associated with high levels of amino acids and fatty acids although the underlying mechanisms for the observed shifts are still unclear. The observed increased levels of metabolites may be due to keratinocyte hyperproliferation, elevated proteolysis and glycolytic activities, and ROS detoxification in patients with psoriasis. Moreover, the metabolites of ornithine, hypoxanthine, crotonic acid and azelaic acid are valuable for the diagnosis of psoriasis. However, larger and wider-ranging sample cohort are required. In addition, further investigations are required to illuminate the significance of the observed amino acids and fatty acids changes. We can conclude that serum metabolites are sensitively responsive to the health condition and that the metabolites related to glycolysis, protein

biosynthesis, redox balance and anabolic activity may be used either for early diagnosis or as a prognosticator for 'health', while having a genetic background of psoriasis without clinical manifestation.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Metabolites identified in the full-scan sample.

Table S2. Receiver operating characteristic (ROC) curve analysis results for potential biomarkers.

Video S1. Author video.