Metabolic Profile for Prediction of Ischemic Stroke in Chinese Hypertensive Population

Xiaofan Guo, MD,* Zhao Li, MD, PhD,* Ying Zhou, MD,* Shasha Yu, MD, PhD,* Hongmei Yang, MD,* Liqiang Zheng, MD, PhD,† Yamin Liu, MD,‡ and Yingxian Sun, MD, PhD*

Background: Stroke burden is extremely high in Chinese hypertensive population. Novel biomarkers for cardiovascular diseases can be detected by metabolomic profiling of human fluids. We aim to find a panel of distinctive plasma metabolites for predicting incident ischemic stroke in hypertensive patients. *Methods:* This is a nested case-control study from a prospective cohort design. Baseline plasma samples were collected from 66 newly developed ischemic stroke cases and 66 matched controls. Untargeted metabolomics was performed by ultra-high performance liquid chromatography-tandem mass spectrometry, and data were analyzed by multivariate and univariate statistics. *Results:* Plasma metabolite profiles clearly differed between hypertensive patients with incident ischemic stroke and without. A total of 12 metabolites were screened and identified as potential biomarkers. The altered metabolic pathways included retinol metabolism, sphingolipid metabolism, glycerophospholipid metabolism, lysine degradation, tyrosine metabolism, and tryptophan metabolism. For prediction of hypertensive ischemic stroke, the panel of specific metabolomics-based biomarkers provided area under the curve of 0.848 (95% confidence interval: 0.783-0.913). *Conclusions:* Our study identified a metabolic signature of incident ischemic stroke in hypertension. Differences in small-molecule metabolites hold translational value in prediction and provide insights into potential new mechanisms of this condition.
Key Words: Hypertension—ischemic stroke—metabolomics—prediction

Introduction

Stroke is a major public health problem, leading to significant morbidity and premature mortality. Globally, stroke is the second leading cause of total years of life lost after ischemic heart disease.[1] However, in China, stroke has become the first leading cause of death in recent years.[2,3] Latest data shows stroke burden in China has increased over the past 30 years, with a prevalence of 1596/100,000 people and an incidence of 345.1/100,000 person-years in 2013.[4] The incidence of first-ever stroke increased annually by 11.9%.[5] It is estimated that there are currently 70 million patients suffering from stroke. The direct stroke-related cost increased from 8.5 billion in 1993 to 103.1 billion in 2008.

Hypertension is the most important risk factor for all types of stroke, especially in China. For each increase of 10 mmHg in systolic blood pressure, there was a 1.44-fold risk for ischemic stroke in Chinese hypertensive patients. Hypertension was associated with a risk ratio of 5.8 for

From the *Department of Cardiology, the First Hospital of China Medical University, Shenyang, Liaoning, China; †Department of Clinical Epidemiology, Library, and Health Policy and Hospital Management, Shengjing Hospital of China Medical University, Shenyang, Liaoning, China; and ‡Division of Cardiology, Department of Medicine, University of California, San Francisco, San Francisco, California.

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Address correspondence to Yingxian Sun, MD, PhD, Department of Cardiology, the First Hospital of China Medical University, 155 Nanjing North Street, Heping District, Shenyang 110001, China. E-mail: yxsun@cmu.edu.cn.

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ischemic stroke among Chinese compared with that of 1.93 among Caucasians.[6] China holds the largest population of hypertension, approaching 250 million estimated recently. There are another 435 million who have prehypertension according to the Chinese guideline.[7] Considering the huge base of hypertensive population, burden of stroke is sharply magnified. Therefore, as highlighted by Chinese guideline, hypertensive population is the target population for prevention of stroke in China.

Conventional risk factors fail to fully predict stroke in hypertension. Accumulating evidence find thousands of small-molecule metabolites in diverse biological systems contributing to complex human diseases. As technology develops, metabolomics becomes a promising tool to gain new insights into early metabolic alterations in cardiovascular disease (CVD).[8] Several studies revealed change of metabolic profile in hypertension or stroke.[9-14] For example, Menni et al found hexadecanedioate was significantly associated with blood pressure and future mortality through analysis of 280 blood metabolites, indicating a novel pathway of blood pressure regulation.[9] Jové et al identified candidate biomarkers form LysoPC family for both stroke recurrence prediction and large-artery atherosclerosis detection.[10] However, limited data are available for prediction of ischemic stroke in hypertensive patients, especially in Chinese population. Here, we used metabolomics to identify novel metabolic signatures for incident ischemic stroke in Chinese hypertensive participants.

Methods

Study Population

Participants included in the current study were selected from Northeast China Rural Cardiovascular Health Study, a rural community-based prospective cohort study designed to identify trend and novel risk factors of CVD in rural China. A detailed description for the study design and methods has been reported previously.[15,16] Briefly, a total of 11,956 participants aged $\geq$35 years were recruited and examined in 2012-2013. Data on demographic characteristics, lifestyle risk factors, dietary habits, family income, and history of cardiovascular disease were obtained by interview with a standardized questionnaire. Fasting blood samples were collected in the morning after at least 12 hours of fasting for all participants, and stored in −80°C refrigerator. We measured metabolic profile of the included patients using these baseline blood samples to explore novel biomarkers for prediction of their future stroke cases. The study was approved by the Ethics Committee of China Medical University (Shenyang, China). All procedures were performed in accordance with the ethical standards.

Participants included in the present analysis have to meet the following criteria: (1) attended examinations at both baseline (2012-2013) and follow-up (2016-2017); (2) had uncontrolled hypertension (systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg) at baseline; (3) were free of CVD (stroke, coronary heart disease, and heart failure) at baseline, and (4) had available plasma sample at baseline for the analysis of metabolomics.

The endpoint of the present study is ischemic stroke. According to the WHO Multinational Monitoring of Trends and Determinants in Cardiovascular Disease criteria,[17,18] stroke events were defined as rapidly developing signs of focal (or global) disturbance of cerebral function lasting >24 hours (unless interrupted by surgery or death) with no apparent nonvascular causes. The definition included patients presenting with clinical signs and symptoms of subarachnoid hemorrhage, intracerebral hemorrhage, thrombosis, and embolism. Ischemic stroke was defined as stroke events with diagnosis of thrombosis or embolism. Transient ischemic attack and chronic cerebral vascular disease were not included. All materials were independently reviewed by the endpoint assessment committee, whose members were all blinded to the study participants' baseline risk factor information.

The median follow-up period is 4.52 years. A total of 4042 participants met all of the criteria. Newly developed ischemic stroke cases during this follow-up period were collected. For this study, 66 incident cases were randomly selected from participants who newly developed ischemic stroke. We then randomly selected controls who remained free from ischemic stroke and matched them to the stroke cases in a 1:1 ratio, according to age (within 3 year), sex, location of the patients, and hypertension grades. The current metabolomics analysis measured metabolite levels of 66 incident cases and 66 control subjects using their stored blood samples at baseline in 2012-2013. Figure 1 shows the sample size flow in our study.

Sample Extraction

Plasma was prepared and thawed on ice-cold condition. After thawing, 100 μL sample was added into an EP tube with total volume of 1.5 mL. Next, 400 μL acetonitrile with low-temperature was added for deprotein purpose, vortexing for 4 minutes at 4°C and standing for 10 minutes at room temperature. And then, it was separated through centrifugation at 14,000 g for 15 minutes at 4°C. Two EP tubes were used to collect the supernatants with 200 μL each of them. The supernatant was lyophilized and stored at −20°C for ultra-high performance liquid chromatography-tandem mass spectrometry (MS) analysis, which was dissolved by using 100 μL of 20% methanol/water solution before experimental injection.

Ultra-High Performance Liquid Chromatography-Tandem MS Metabolomics Analysis

Untargeted metabolomics was performed on a Waters UPLC system (Waters Company, MA) coupled online to a

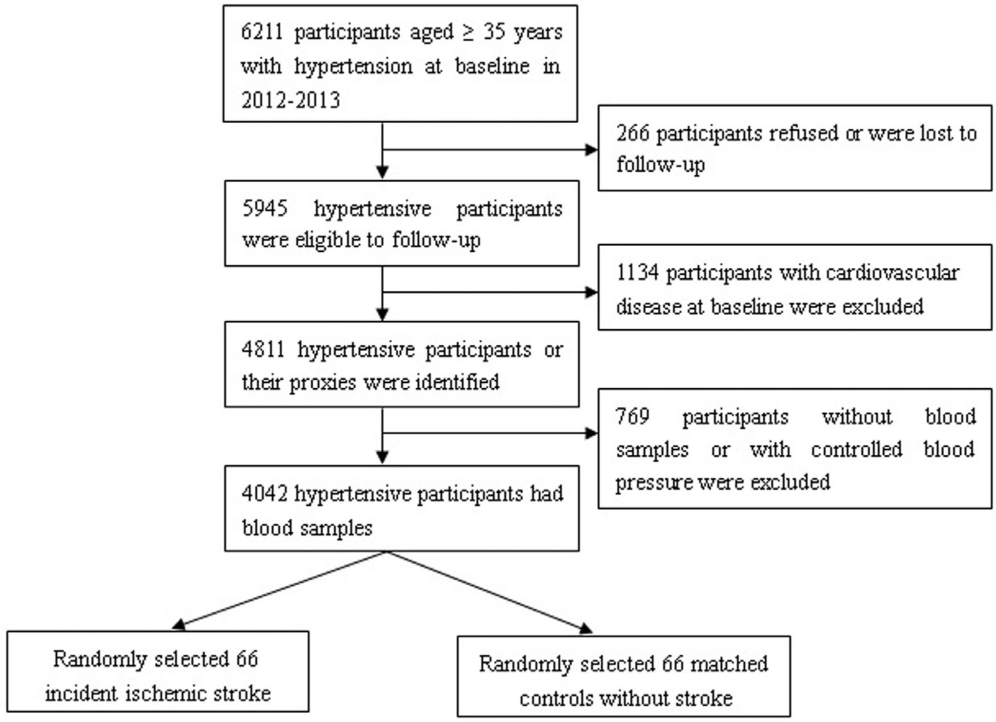

Figure 1. *Flowchart of participant selection of the study.*

Q Exactive mass spectrometer (Thermo Fisher Scientific). At positive mode, samples were resolved over a BEH C8 column (1.7 μm, 2.1 × 100 mm) at 50°C using mobile phases A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid) at a flow rate of 350 μL/min. The gradient starts from 5% B and uniformly increases to 100% B with 24 minutes, and keeps 3.5 minutes for equilibrium. After this, phase B was decreased to 5% with total time attaining to 30 minutes. To MS analysis, the conditions were used as follows: Spray Voltage (kV): 3.8; Capillary temperature (°C): 320; Aux gas heater temperature (°C): 350; Sheath gas flow rate (Arb): 35; Aux gas flow rate (Arb): 8; S-lens RF level: 50; Mass range (m/z): 70-1000, Full ms resolution: 70000; MS/MS resolution:17500; TopN: 8; NCE/stepped NCE: 20, 40.

At negative mode, samples were resolved over a BEH T3 column (1.8 μm, 2.1 × 100 mm) at 50°C using mobile phases A (water with 6.5 mM ammonium bicarbonate) and B (95% methanol/water solution with 6.5 mM ammonium bicarbonate) at a flow rate of 350 μL/min. The gradient starts from 5% B and uniformly increases to 100% B with 18 minutes, and keeps 4 minutes for equilibrium. After this, phase B was decreased to 5% with total time attaining to 25 minutes. To MS analysis, the conditions were used as follows: Spray Voltage (kV): −3.0; Capillary temperature (°C): 320; Aux gas heater temperature (°C): 350; Sheath gas flow rate (Arb): 35; Aux gas flow rate (Arb): 8; S-lens RF level: 50; Mass range (m/z): 70-1000, Full ms resolution: 70000; MS/MS resolution:17500; TopN: 8, NCE/stepped NCE: 20,40.

Statistical Analysis

The raw data were processed by XCMS package. Data pretreatment procedures, such as peak discrimination, filtering, alignment, and matching were performed to generate a multivariable data matrix of sample identity, ion identity, and ion abundance. The 80% rule was used to treat the missing values for each sample group. The systematic and automated approach and homemade software (OSI/SMMS) was also used to optimize identification of the metabolites.[19] Competitive adaptive reweighted sampling algorithm was adopted to effectively select the optimal combination of variables.[20,21]

For multivariate statistical analysis, the normalized data were imported to SIMCA software (version 13.0, Umetrics, Sweden). First, principal component analysis, an unsupervised pattern recognition approach, was used to observe any intrinsic clusters between case and control groups. Then, partial least squares discriminant analysis (PLS-DA) and orthogonal partial least-squared discriminant analysis (OPLS-DA) were performed to obtain better class separation in a supervised manner. Supervised methods are frequently used in metabolomics to identify metabolic signatures that relate to the variable of interest while reducing the effects of the other sources of variance. PLS measures how much a variable contributes to the discrimination of the different groups, while OPLS model evolved from PLS model and factorizes the data variance into a first component which relates to the variable of interest and a second uncorrelated component (ie, orthogonal). Performances of the 2 models are similar.[22,23] R^2Y (cum) was calculated to estimate the "goodness of fit" of the model, and Q^2 (cum) to estimate the ability of prediction. For univariate statistical analysis, data following normal distribution were compared by Student's *t*-test, while nonparametric test was performed on the data deviating from normal distribution by Wilcoxon Mann−Whitney test. Ions with variable influence on projection values >1.0 and P values of < .05 were identified as potential differential metabolites. Heat maps and hierarchical cluster analyses were conducted using R package. Open database sources, including the Kyoto Encyclopedia of Genes and Genomes pathway database and MetaboAnalyst were used to identify metabolic pathways. The area under the curve in receiver operating characteristic analysis was calculated to evaluate the discriminating power of the biomarkers. Statistical analyses were performed using SPSS software version 19.0 (IBM Corp., Armonk, NY). A P value < .05 was considered statistically significant.

Results

Untargeted metaboliomics were performed in a total of 132 hypertensive participants in the present study, as shown in Figure 1. Baseline characteristics are shown in Table 1. Both case group and control group had similar percentages of sex, smoking and drinking status, as well as blood pressure grades. There were no significant differences in body mass index, levels of glucose and lipids between case group and control group (all $P > .05$). Compared with control subjects, patients with incident ischemic stroke had higher levels of waist circumference.

Figure 2 shows the representative LC-MS total ion chromatogram of the serum samples in both groups. The spectral peak of each metabolite is optimal and the peaks are well separated from each other. Visible difference between cases and controls were observed. Among the 155 ion selected, there were 119 positive ion and 36 negative ions.

In the preliminary analysis of these data, principal component analysis was employed to explore different clustering in the two groups. A trend of separation was observed between groups (Fig 3A). Subsequently, we used PLS-DA and OPLS-DA models to characterize the metabolic disturbances. PLS-DA model improved the clustering segregation between the two groups with cumulative R^2Y at 0.896 and Q^2 at 0.584 (Fig 3B and C). Clear differences were obtained for incident ischemic stroke cases versus controls (cumulative R^2Y at 0.948 and Q^2 at 0.787) with statistical validation in OPLS-DA model (Fig 4A and B). Potential differential metabolites were also screened by the S-plot of the established OPLS-DA model. The distance from the biomarkers to the center of the S-plot determines the contribution of metabolites to the class discrimination (Fig 4C).

As summarized in Table 2, a panel of 12 endogenous ions with variable influence on projection values > 1.0 from PLS-DA model and $P < 0.05$ from univariate statistical analysis was considered the potential differential metabolites. Figure 5 is a heat map showing the average normalized quantities of the 12 differential metabolites in case and control groups. To determine the possible metabolic pathway that was disrupted in ischemic stroke, all the differential metabolites were analyzed using Kyoto Encyclopedia of Genes and Genomes and MetaboAnalyst. The potential pathways included retinol metabolism, sphingolipid metabolism, glycerophospholipid metabolism, lysine degradation, tyrosine metabolism and tryptophan metabolism (Fig 6). For prediction of hypertensive ischemic stroke, the corresponding receiver operating characteristic curve of the combined differential

Table 1. *Baseline characteristics of hypertensive participants with incident ischemic stroke or not*

Variables	Cases (n = 66)	Controls (n = 66)	*P* value
Age (year)	60.8 ± 8.1	60.7 ± 8.3	.913
Male	41 (62.1)	41 (62.1)	—
BMI(kg/m^2)	25.9 ± 3.6	25.0 ± 2.9	.102
WC (cm)	88.8 ± 13.8	84.8 ± 8.4	.047
TC (mmol/L)	5.3 ± 1.0	5.2 ± 1.1	.891
TG (mmol/L)	2.1 ± 2.4	2.1 ± 1.9	.927
LDL-C (mmol/L)	3.0 ± 0.9	2.9 ± 0.8	.55
HDL-C (mmol/L)	1.4 ± 0.4	1.5 ± 0.4	.079
FPG (mmol/L)	6.1 ± 1.4	6.3 ± 3.1	.647
Blood pressure			—
Grade 1	27 (40.9)	27 (40.9)	
Grade 2	20 (30.3)	20 (30.3)	
Grade 3	19 (28.8)	19 (28.8)	
Current smoking status	34 (51.5)	36 (54.5)	0.727
Current drinking status	29 (43.9)	25 (37.9)	0.479

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

Data are expressed as the mean ± SD or as n (%).

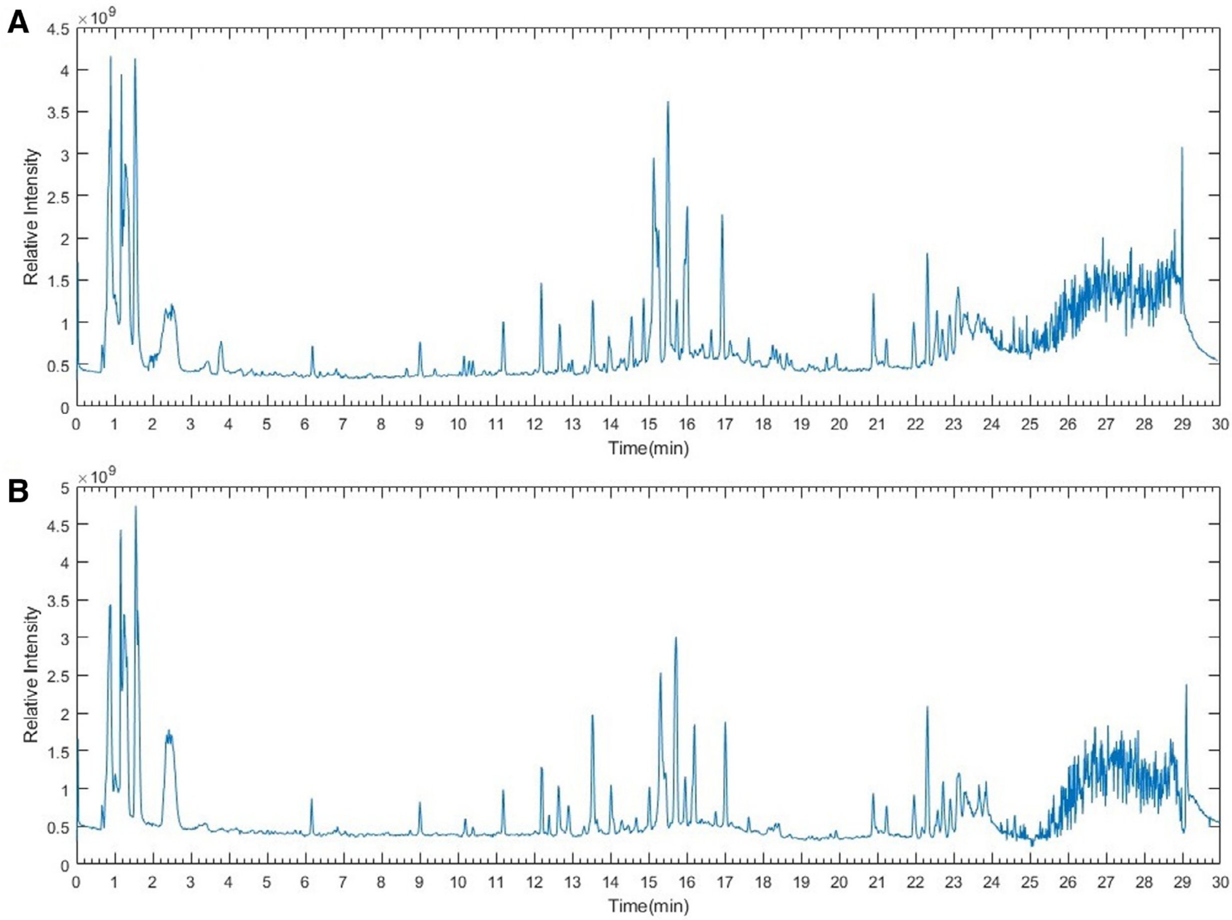

Figure 2. *Representative total ion chromatograms of hypertensive patients with incident ischemic stroke (A) and without (B).*

biomarkers had an area under the curve of 0.848 (95% confidence interval: 0.783-0.913), as shown in Figure 7.

Discussion

Our study described a comprehensive metabolomic profile of incident stroke in Chinese hypertensive patients. Untargeted metabolomics revealed significant pattern change between hypertensive patients with stroke event or without, indicating an involvement of metabolic disturbance in ischemic stroke. The metabolic pathways of these biomarkers were disturbed, especially for sphingolipid metabolism. Combinations of metabolic biomarkers provided favorable predictive values for ischemic stroke in this population.

As technology develops, metabolomics becomes an important tool for cardiovascular and cerebrovascular diseases. Accumulating evidence suggests that metabolic

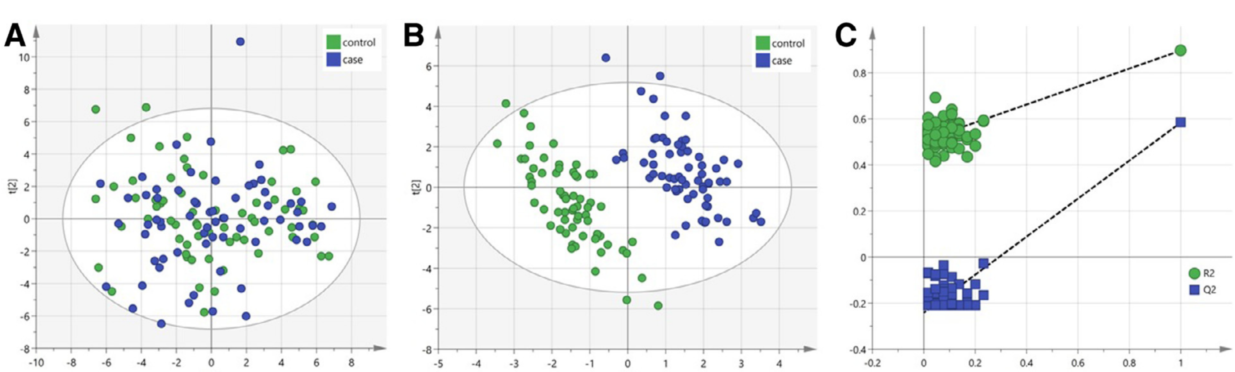

Figure 3. *Multivariate statistical analysis of serum metabolic profiling in hypertensive patients with incident ischemic stroke (case) and without (control). (A) Principal component analysis (PCA) score plot; (B) partial least squares discriminant analysis (PLS-DA) score plots; (C) statistical validation of established PLS-DA model with permutation analysis (100 random permutations).*

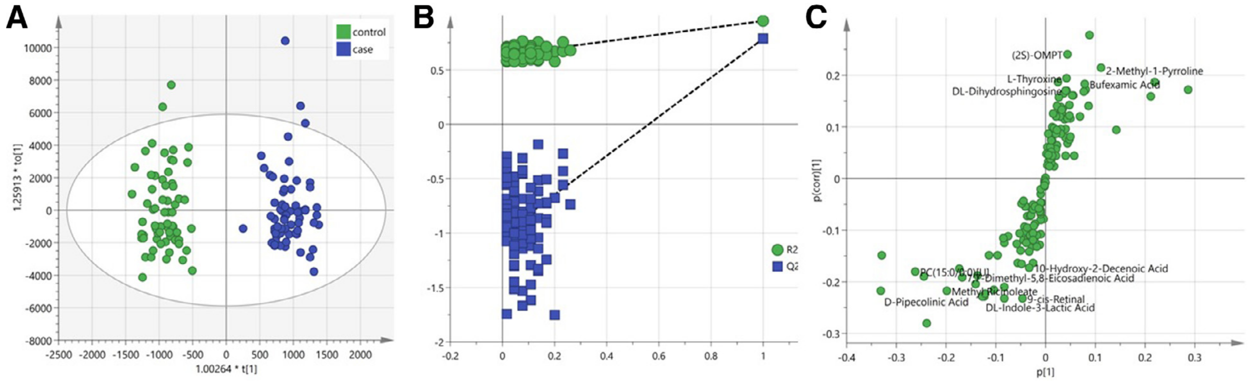

Figure 4. *Multivariate statistical analysis of serum metabolic profiling in hypertensive patients with incident ischemic stroke (case) and without (control). (A) Orthogonal projection to latent structure-discriminant analysis (OPLS-DA) score plots; (B) statistical validation of established OPLS-DA model with permutation analysis (100 random permutations); (C) S-plot of established OPLS-DA model.*

Table 2. *Potential plasma biomarkers for ischemic stroke in hypertensive patients*

Metabolites	Retention time (min)	Mass-to-charge ratio	VIP value	Fold change	*P* value
9-cis-Retinal	18.77	285.220	1.962	0.807	.003
DL-Indole-3-Lactic Acid	5.85	206.080	1.769	0.792	.005
Methyl Ricinoleate	18.12	313.272	1.750	0.795	.016
(2S)-OMPT	12.60	465.249	1.740	0.787	.007
2-Methyl-1-Pyrroline	1.02	84.081	1.674	1.307	.021
D-Pipecolinic Acid	0.92	130.086	1.560	0.758	.024
7,7-Dimethyl-5,8-Eicosadienoic Acid	18.63	335.295	1.468	1.433	.032
PC(15:0/0:0)[U]	14.75	482.322	1.446	0.728	.024
DL-Dihydrosphingosine	13.81	302.304	1.426	1.116	.031
Bufexamic Acid	5.10	224.143	1.414	1.072	.034
10-Hydroxy-2-Decenoic Acid	10.47	185.117	1.394	1.137	.038
L-Thyroxine	10.33	777.690	1.372	1.116	.042

VIP, variable influence on projection.

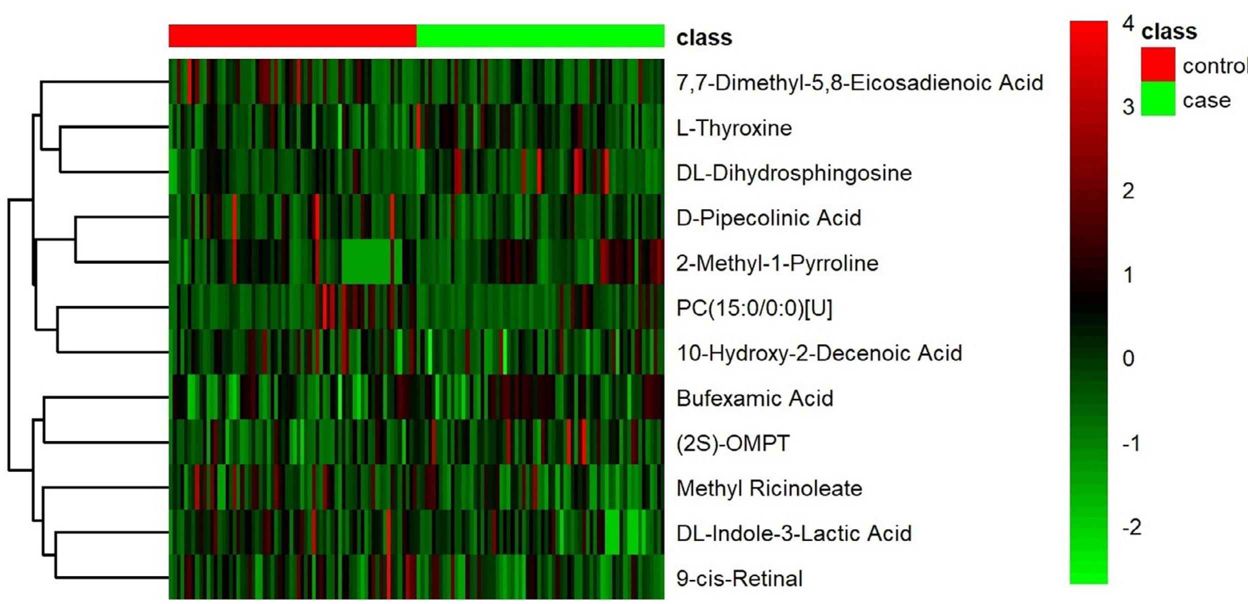

Figure 5. *Heatmap of differential serum metabolites between hypertensive patients with incident ischemic stroke and those without.*

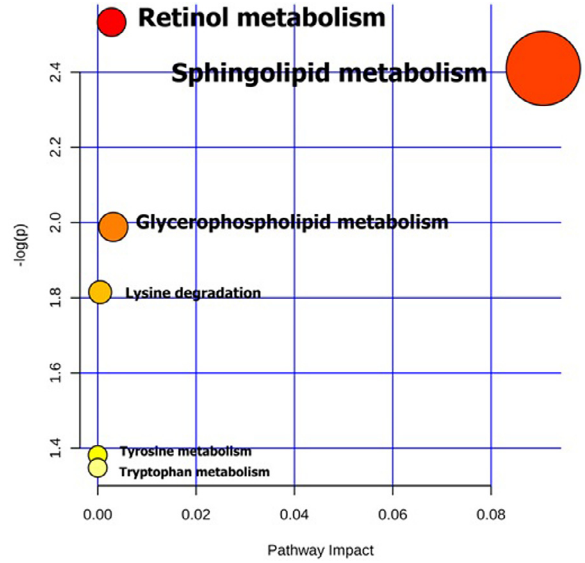

Figure 6. *Disturbed metabolic pathways. The x-axis represents the pathway impact, and the y-axis represents the −log (p).*

profile is promising for predicting CVDs in different populations.[24-26] In hypertension research, Akira et al compared metabolic profiles of spontaneously hypertensive rats with those of their age-matched normotensive Wistar Kyoto rats. The researchers found urine levels of citrate and a-ketoglutarate were decreased in spontaneously hypertensive rats, suggesting an involvement of metabolic changes with blood pressure regulation.[27] Profound metabolic differences between hypertensive and normotensive rat models were also found in blood samples.[28] Furthermore, the urinary levels of taurine and creatine were found to be higher in stroke-prone spontaneously hypertensive rats than those in the Wistar Kyoto rats, implying a potential usage of metabolomics in hypertension related stroke.[29]

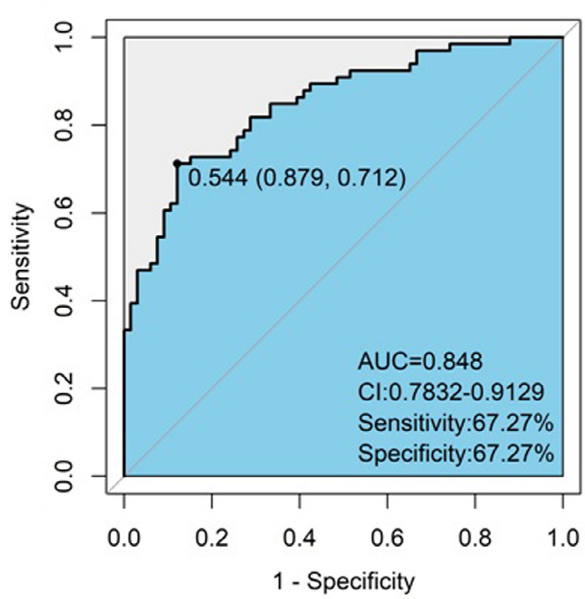

Figure 7. *Receiver operating characteristic curves of the biomarker panel to predict ischemic stroke.*

Previous study found significant change of blood metabolites in acute ischemic stroke model.[30] Wang et al performed metabolomics in 40 acute ischemic stroke patients and 29 matched controls. Tyrosine, lactate, and tryptophan were shown as a panel of potential biomarkers of acute ischemic stroke.[31] Purroy et al also found specific metabolomic profiles associated with representative neuroimaging features in patients with transient ischemic attack.[14] However, features and mechanism of hypertensive stroke are different from nonhypertensive stroke.[32,33] Especially in China, where hypertension is associated with much higher risk of stroke than other complications, such as ischemic heart disease. The present study for the first time provided data on metabolic profile of Chinese hypertensive stroke.

In the current study, we found a panel of metabolites was significantly regulated in hypertensive patients who developed ischemic stroke. Most of the metabolites are first found related to stroke and the mechanism is unclear. For example, indolelactic acid was observed to be associated with cancer in previous papers.[34,35] The pathophysiological effect of indolelactic acid has not been studied yet. Pipecolic acid is a metabolite of lysine, possibly relating to some complex diseases such as pyridoxine-dependent epilepsy.[36,37] Ferrarini et al reported an increased level of pipecolic acid in severe sleep apnea and hypopnea syndrome when compared to nonsevere patients.[38] Our study opened new lines of research that will benefit our understanding of hypertensive stroke. The effects of these metabolites on human body need further investigations.

The present study had several limitations. First, our sample size was relatively small, and further validation in a larger population is expected. Second, interindividual metabolic variability of subjects was inevitable because metabolic activity is susceptible to various factors such as environment and lifestyles. Additionally, other classes of lipids and some isomers of the metabolites might not be identified in the current study.

In conclusion, untargeted metabolomic studies are a useful tool in detecting abnormalities of complicated diseases. The plasma metabolic profile between incident ischemic stroke cases and nonstroke controls in hypertensive patients were significantly different. Combinations of metabolic biomarkers provided optimal predictive values for hypertensive ischemic stroke. A better understanding of the underlying mechanisms might identify novel therapeutic targets to prevent this unmet medical condition.

Conflict of Interest

The authors have no conflict of interests to disclose.

References

1. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of

death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390:1151-1210.
2. Liu L, Wang D, Wong KS, et al. Stroke and stroke care in China: huge burden, significant workload, and a national priority. Stroke 2011;42:3651-3654.
3. Zhou M, Wang H, Zhu J, et al. Cause-specific mortality for 240 causes in China during 1990-2013: a systematic subnational analysis for the Global Burden of Disease Study 2013. Lancet 2016;387:251-272.
4. Wang W, Jiang B, Sun H, et al. Prevalence, incidence, and mortality of stroke in China: results from a nationwide population-based survey of 480 687 adults. Circulation 2017;135:759-771.
5. Ning X, Sun J, Jiang R, et al. Increased stroke burdens among the low-income young and middle aged in rural China. Stroke 2017;48:77-83.
6. Zhang XF, Attia J, D'Este C, et al. The relationship between higher blood pressure and ischaemic, haemorrhagic stroke among Chinese and Caucasians: meta-analysis. Eur J Cardiovasc Prev Rehabil 2006;13:429-437.
7. Wang Z, Chen Z, Zhang L, et al. Status of hypertension in China: results from the China hypertension survey, 2012-2015. Circulation 2018;137:2344-2356.
8. Cheng S, Shah SH, Corwin EJ, et al. Potential impact and study considerations of metabolomics in cardiovascular health and disease: a scientific statement from the American Heart Association. Circ Cardiovasc Genet 2017;10(2): e000032. https://doi.org/10.1161/HCG.0000000000000032.
9. Bagherniya M, Khayyatzadeh SS, Heidari Bakavoli AR, et al. Serum high-sensitive C-reactive protein is associated with dietary intakes in diabetic patients with and without hypertension: a cross-sectional study. Ann Clin Biochem 2018;55:422-429.
10. Jové M, Mauri-Capdevila G, Suárez I, et al. Metabolomics predicts stroke recurrence after transient ischemic attack. Neurology 2015;84:36-45.
11. Hao Y, Wang Y, Xi L, et al. A Nested Case-Control Study of association between metabolome and hypertension risk. Biomed Res Int 2016;2016:7646979.
12. Zheng Y, Yu B, Alexander D, et al. Metabolomics and incident hypertension among blacks: the atherosclerosis risk in communities study. Hypertension 2013;62:398-403.
13. Kulkarni H, Meikle PJ, Mamtani M, et al. Plasma lipidomic profile signature of hypertension in Mexican American families: specific role of diacylglycerols. Hypertension 2013;62:621-626.
14. Purroy F, Cambray S, Mauri-Capdevila G, et al. Metabolomics predicts neuroimaging characteristics of transient ischemic attack patients. EBioMedicine 2016;14:131-138.
15. Li Z, Guo X, Zheng L, et al. Prehypertension in rural northeastern China: results from the northeast China rural cardiovascular health study. J Clin Hypertens (Greenwich) 2014;16:664-670.
16. Li Z, Guo X, Zheng L, et al. Grim status of hypertension in rural China: results from Northeast China Rural cardiovascular health study 2013. J Am Soc Hypertens 2015;9:358-364.
17. WHO MONICA project MONICA Manual. Available at: http://www.ktl.fi/publications/monica. URNLNBN:fife 19981147, 1990.
18. Zhao D, Liu J, Wang W, et al. Epidemiological transition of stroke in China: twenty-one-year observational study from the Sino-MONICA-Beijing Project. Stroke 2008;39:1668-1674.
19. Zhao X, Zeng Z, Chen A, et al. Comprehensive strategy to construct in-house database for accurate and batch identification of small molecular metabolites. Anal Chem 2018;90:7635-7643.
20. Li H, Liang Y, Xu Q, et al. Key wavelengths screening using competitive adaptive reweighted sampling method for multivariate calibration. Anal Chim Acta 2009;648:77-84.
21. Tan BB, Liang YZ, Yi LZ, et al. Identification of free fatty acids profiling of type 2 diabetes mellitus and exploring possible biomarkers by GC−MS coupled with chemometrics. Metabolomics 2010;6:219-228.
22. Worley B, Powers R. Multivariate analysis in metabolomics. Curr Metabolomics 2013;1:92-107.
23. Alonso A, Marsal S, Julià A. Analytical methods in untargeted metabolomics: state of the art in 2015. Front Bioeng Biotechnol 2015;3:23.
24. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57-63.
25. Kalim S, Clish CB, Wenger J, et al. A plasma long-chain acylcarnitine predicts cardiovascular mortality in incident dialysis patients. J Am Heart Assoc 2013;2:e000542.
26. Rizza S, Copetti M, Rossi C, et al. Metabolomics signature improves the prediction of cardiovascular events in elderly subjects. Atherosclerosis 2014;232:260-264.
27. Akira K, Masu S, Imachi M, et al. 1H NMR-based metabonomic analysis of urine from young spontaneously hypertensive rats. J Pharm Biomed Anal 2008;46:550-556.
28. Lu YAJ, Wang G, et al. Gas chromatography/time-of-flight mass spectrometry based metabonomic approach to differentiating hypertension- and age-related metabolic variation in spontaneously hypertensive rats. Rapid Commun Mass Spectrom 2008;22:2882-2888.
29. Akira K, Imachi M, Hashimoto T. Investigations into biochemical changes of genetic hypertensive rats using 1H nuclear magnetic resonance-based metabonomics. Hypertens Res 2005;28:425-430.
30. Su L, Zhao H, Zhang X, et al. UHPLC-Q-TOF-MS based serum metabonomics revealed the metabolic perturbations of ischemic stroke and the protective effect of RKIP in rat models. Mol Biosyst 2016;12:1831-1841.
31. Wang D, Kong J, Wu J, et al. GC-MS-based metabolomics identifies an amino acid signature of acute ischemic stroke. Neurosci Lett 2017;642:7-13.
32. Arboix A, Roig H, Rossich R, et al. Differences between hypertensive and non-hypertensive ischemic stroke. Eur J Neurol 2004;11:687-692.
33. Yu JG, Zhou RR, Cai GJ. From hypertension to stroke: mechanisms and potential prevention strategies. CNS Neurosci Ther 2011;17:577-584.
34. Zhang T, Wu X, Ke C, et al. Identification of potential biomarkers for ovarian cancer by urinary metabolomic profiling. J Proteome Res 2013;12:505-512.
35. Cala MP, Agulló-Ortuño MT, Prieto-García E, et al. Multiplatform plasma fingerprinting in cancer cachexia: a pilot observational and translational study. J Cachexia Sarcopenia Muscle 2018;9:348-357.
36. Plecko B, Stöckler-Ipsiroglu S, Paschke E, et al. Pipecolic acid elevation in plasma and cerebrospinal fluid of two patients with pyridoxine-dependent epilepsy. Ann Neurol 2000;48:121-125.
37. Wangler MF, Hubert L, Donti TR, et al. A metabolomic map of Zellweger spectrum disorders reveals novel disease biomarkers. 2018 (Feb 8). https://doi.org/10.1038/gim.2017.262.
38. Ferrarini A, Rupérez FJ, Erazo M, et al. Fingerprinting-based metabolomic approach with LC-MS to sleep apnea and hypopnea syndrome: a pilot study. Electrophoresis 2013;34:2873-2881.