

Serum metabolomic signatures associated with gastric signet-ring cell carcinoma: A dual-platform mass spectrometry study

HUILING CAO¹, SHUWEI GUO¹, JINCHANG LI¹, YUYING BIAN¹, JIANJIANG SHEN¹,
JIA WANG¹, FEI MENG¹, WENLU SUN¹, QINGMIN SUN² and WANJIAN GU^{1,3}

¹Department of Clinical Laboratory, Affiliated Hospital of Nanjing University of Chinese Medicine, Jiangsu Province Hospital of Chinese Medicine, Nanjing, Jiangsu 210029, P.R. China; ²Jiangsu Province Key Laboratory of Tumor Systems Biology and Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Jiangsu Province Hospital of Chinese Medicine, Nanjing, Jiangsu 210029, P.R. China; ³Department of Clinical Laboratory, The Affiliated People's Hospital of Kizilsu Kirghiz Autonomous Prefecture, Nanjing Medical University, Artux, Xinjiang Uyghur Autonomous Region 845350, P.R. China

Received March 28, 2026; Accepted July 22, 2026

DOI: 10.3892/ol.2026.15900

Abstract. Gastric signet-ring cell carcinoma (GSRCC), a distinct subtype of gastric adenocarcinoma, is associated with increasing incidence and poor prognosis. The metabolic landscape remains poorly understood and non-invasive biomarkers for early detection are limited. The present study aimed to explore the metabolic signatures for GSRCC using metabolomics profiling. Serum samples from 8 treatment-naïve patients with GSRCC and 10 healthy controls were analyzed using liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. Differential metabolites were identified through multivariate statistical analysis (principal component analysis, partial least squares discriminant analysis and orthogonal partial least squares discriminant analysis) with a criteria of variable importance in projection >1, $q < 0.05$ (Benjamini-Hochberg false discovery rate correction) and $\log_2$ fold change >0.58. Metabolic pathway enrichment was performed using the Kyoto Encyclopedia of Genes and

Genomes database. Key metabolites associated with routine clinical biochemistry parameters were analyzed in an independent cohort (35 patients with GSRCC and 35 controls). A total of 68 differential metabolites were identified (25 upregulated and 43 downregulated), predominantly comprising of phospholipids and amino acids. Pathway enrichment revealed significant alterations in 'choline metabolism in cancer', 'glycerophospholipid metabolism', 'pentose and glucuronate interconversions' and 'arginine biosynthesis' (all $P < 0.05$; $q < 0.05$). Among the enriched pathway-associated metabolites, phosphatidylcholine [PC(20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z))] and dihydroxyacetone phosphate (DHAP) acyl ester showed the most significant differences ($q < 0.05$) and demonstrated area under the curve values exceeding 0.80 (0.89 and 0.99, respectively). Patients with GSRCC exhibited significantly lower levels of cholinesterase, total protein, albumin and hemoglobin compared with controls (all $P < 0.01$). Overall, the present study identified serum metabolomic alterations in GSRCC, highlighting the key role of choline and glycerophospholipid metabolism, with PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] and DHAP acyl ester representing exploratory candidate metabolic signatures associated with GSRCC, providing a foundation for future biomarker discovery.

Correspondence to: Dr Wanjian Gu, Department of Clinical Laboratory, Affiliated Hospital of Nanjing University of Chinese Medicine, Jiangsu Province Hospital of Chinese Medicine, 155 Hanzhong, Qinhuai, Nanjing, Jiangsu 210029, P.R. China
E-mail: arrowsgu@163.com

Abbreviations: GSRCC, gastric signet-ring cell carcinoma; LC-MS, liquid chromatography-mass spectrometry; GC-MS, gas chromatography-mass spectrometry; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in projection; KEGG, Kyoto Encyclopedia of Genes and Genomes; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DHAP, dihydroxyacetone phosphate; ROC, receiver operating characteristic; AUC, area under the curve

Key words: gastric signet-ring cell carcinoma, metabolomics, exploratory metabolic signatures, choline metabolism, glycerophospholipid metabolism, mass spectrometry

Introduction

Gastric cancer ranks as the third most prevalent malignancy in China with regard to both incidence and mortality, accounting for 44.0% of new GC cases and 48.6% of GC-related deaths worldwide (1,2). Despite the overall incidence of gastric cancer having declined in recent decades, the proportion of gastric signet-ring cell carcinoma (GSRCC), a distinct histopathological subtype, has exhibited an increasing trend (3). GSRCC is characterized by abundant intracytoplasmic mucin that displaces the nucleus to the periphery, creating the classic 'signet-ring' morphology. According to the World Health Organization classification, gastric adenocarcinoma with signet-ring cell component of >50% is defined as GSRCC, which falls under the category of poorly cohesive

carcinomas (4). Due to the marked heterogeneity of gastric cancer, pure GSRCC is relatively uncommon in clinical practice (5), with mixed adenocarcinomas containing signet-ring cell components being more frequently encountered.

GSRCC is associated with aggressive biological behavior, including high propensity for lymph node metastasis and distant dissemination, contributing to unfavorable prognosis (6). Notably, early-stage GSRCC demonstrates markedly lower metastatic potential and more favorable response to curative treatment, underscoring the importance of early detection (7). Currently, the most established diagnostic method for GSRCC is histopathological examination of endoscopic biopsy specimens, an invasive procedure with inherent limitations including patient discomfort, sampling difficulty and operational complexity (8).

Metabolomics, the comprehensive analysis of small-molecule metabolites in biological systems, has emerged as an important tool in cancer biomarker discovery (9). Circulating metabolites reflect the systemic metabolic reprogramming associated with tumorigenesis and progression, offering potential for non-invasive diagnostic applications. Recent metabolomic studies have identified candidate biomarkers for a number of malignancies, including hypoxanthine for lung adenocarcinoma (10), pimelic acid and L-serine for papillary thyroid carcinoma (11), hyaluronan and proteoglycan link protein-1 for peritoneal metastasis in pancreatic ductal adenocarcinoma (12). However, systematic metabolomic profiling of GSRCC remains limited. This is largely due to the rarity of this subtype (3-5% of gastric cancers) (5), which limits the availability of large sample cohorts, as well as the technical challenges posed by its mucinous composition. These factors underscore the need for and value of the present study.

In the present study, a dual-platform approach integrating liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) was employed to comprehensively characterize the serum metabolomic profile of patients with GSRCC compared with healthy controls. The objectives were to identify differential metabolites associated with GSRCC, elucidate perturbed metabolic pathways and evaluate the exploratory candidate metabolic signatures.

Materials and methods

Study population and sample collection. Serum samples were prospectively collected from patients diagnosed with GSRCC at Jiangsu Province Hospital of Chinese Medicine (Nanjing, China) between March 2023 and November 2024. Inclusion criteria were as follows: i) Histopathologically determined GSRCC based on surgical specimens; ii) treatment-naïve status with no prior anticancer therapy (including surgery, chemotherapy, radiotherapy or targeted therapy); iii) age ≥ 18 years; iv) availability of complete clinical and pathological data, including TNM stage, tumor location and lymph node metastasis status; and v) absence of major underlying diseases (such as severe cardiovascular, hepatic, renal or autoimmune disorders) and no history of other malignancies. Exclusion criteria included: i) Any prior surgical treatment; ii) neoadjuvant therapy; iii) presence of other malignancies; or iv) concurrent inflammatory or metabolic disorders. The discovery cohort comprised 8 patients with GSRCC and 10 age- and sex-matched

healthy controls. In the discovery cohort, the age range was 40-75 years for patients with GSRCC and 30-70 years for healthy controls, with 3 men (37.5%) and 5 women (62.5%) in the GSRCC group, and 5 men (50.0%) and 5 women (50.0%) in the control group. An independent validation cohort included 35 patients with GSRCC and 35 healthy controls. In the validation cohort, the age range was 35-84 years for patients with GSRCC and 38-78 years for healthy controls, with 17 men (48.6%) and 18 women (51.4%) in the GSRCC group, and 18 men (51.4%) and 17 women (48.6%) in the control group. All GSRCC diagnoses were determined by histopathological examination of surgical specimens. Clinical and pathological data, including age, sex, tumor location, tumor size, depth of invasion, lymphovascular invasion, perineural invasion and lymph node metastasis, were systematically recorded. Venous blood samples (3 ml) were collected preoperatively, centrifuged at 1,500 $\times$ g, 4°C for 5 min, and serum was aliquoted and stored at -80°C until analysis.

Chemicals and reagents. LC-MS grade methanol, acetonitrile and formic acid were purchased from Thermo Fisher Scientific, Inc. Chloroform (analytical grade) was obtained from Shanghai Titan Scientific Co., Ltd. Hexane (high-performance liquid chromatography grade) was purchased from Shanghai Anpel Experimental Technology Co., Ltd. Pyridine (HPLC grade), O-methylhydroxylamine hydrochloride (97%) and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) were obtained from Shanghai Macklin Biochemical Co., Ltd., Shanghai Aladdin Biochemical Technology Co., Ltd. and Tokyo Chemical Industry Co., Ltd., respectively.

Sample preparation. Serum samples were thawed on ice and 150 μ l aliquots were transferred to microcentrifuge tubes. Protein precipitation was performed by adding 600 μ l methanol-acetonitrile (2:1, v/v) containing a mixture of internal standards (L-2-chlorophenylalanine, succinic acid-d4, L-valine-d8, and cholic acid-d4) at a concentration of 4 μ g/ml. Samples were vortexed at room temperature for 1 min, sonicated in an ice-water bath for 10 min and incubated at -40°C for 2 h. Following centrifugation at 13,000 $\times$ g for 20 min at 4°C, the supernatant was collected and filtered through 0.22 μ m membrane filters. For LC-MS analysis, 150 μ l supernatant was transferred to autosampler vials. For GC-MS analysis, an additional 150 μ l supernatant was evaporated to dryness using a centrifugal concentrator. The residue was derivatized with 80 μ l methoxyamine hydrochloride in pyridine (15 mg/ml) at 37°C for 1 h, followed by addition of 50 μ l BSTFA and 20 μ l hexane containing internal standards, and then incubated at 70°C for 1 h. Quality control samples were prepared by pooling equal volumes of all study samples. The prepared serum samples were sent to Shanghai OE Biotech Co., Ltd. for LC-MS/MS and GC-MS analysis.

LC-MS/MS analysis. LC-MS analysis was performed using a Waters ACQUITY UPLC I-Class PLUS System (Waters Corporation) coupled with a Thermo Scientific™ Q Exactive™ HF mass spectrometer (Thermo Fisher Scientific, Inc.). Chromatographic separation was achieved on an ACQUITY UPLC HSS T3 column (100.0 $\times$ 2.1 mm, 1.8 μ m; Waters Corporation) maintained at 45°C. The mobile phase consisted

of water containing 0.1% formic acid (phase A) and acetonitrile (phase B) at a flow rate of 0.35 ml/min. The injection volume was 3 μ l. The mass spectrometer was operated in both positive and negative ionization modes with full scan range of 70-1050 m/z. The ion source parameters were set as follows: Spray voltage, +3,500 V (positive mode) and -3,000 V (negative mode); capillary temperature, 320°C; auxiliary gas heater temperature, 350°C; sheath gas flow rate, 35 Arb; auxiliary gas flow rate, 8 Arb; and S-lens RF level, 50. The nebulizer pressure was maintained at 45 psi.

GC-MS analysis. GC-MS analysis was conducted using an Agilent 8890-5977B gas chromatograph-mass spectrometer (Agilent Technologies, Inc.) equipped with a DB-5MS capillary column (30 m x 0.25 mm x 0.25 μ m; Agilent Technologies, Inc.). Helium was used as carrier gas at a constant flow rate of 1.0 ml/min. The injector temperature was set at 260°C with splitless injection of 1 μ l. The oven temperature program was as follows: Initial temperature 60°C held for 30 sec, increased to 125°C at 8°C/min, then to 210°C at 8°C/min, then to 270°C at 15°C/min and finally to 305°C at 20°C/min held for 5 min. The ion source temperature was 230°C, quadrupole temperature 150°C and electron energy 70 eV. Mass spectra were acquired in full scan mode (50-500 m/z).

Data processing and metabolite identification. LC-MS/MS and GC-MS datasets were processed and quality-controlled independently. For LC-MS/MS data, raw data were processed using Progenesis QI v3.0 (Nonlinear Dynamics, Ltd.; Waters Corporation) for baseline filtering, peak detection, integration, retention time correction, alignment and normalization and metabolite annotation was performed using the Human Metabolome Database (<https://www.hmdb.ca>), Lipidmaps (v2.3; <https://www.lipidmaps.org>), METLIN database (<http://metlin.scripps.edu>) and the in-house LuMet-Animal 3.0 LC-MS/MS database (Shanghai OE Biotech Co., Ltd.). The qualitative score for LC-MS/MS had a maximum of 80 points, comprising 20 points for accurate mass matching, 20 points for MS/MS fragmentation matching, 20 points for isotope distribution matching and 20 points for retention time matching; higher scores indicated more accurate identification. For GC-MS data, raw data were processed using MS-DIAL v4.24 (http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL) for peak detection, deconvolution, identification, alignment, filtering and missing value imputation and metabolite identification was based on retention time, fragmentation mass spectra and similarity matching against the in-house LuMet-GC 5.0 database (Shanghai OE Biotech Co., Ltd.), with the National Institute of Standards and Technology database (<https://www.nist.gov/>) also used as a reference for auxiliary identification. The qualitative score for GC-MS had a maximum of 100 points. For quality control and filtering, ions with >50% missing values (zero values) within each group were removed and remaining zero values were replaced with half of the minimum intensity value across all samples. For LC-MS/MS data, compounds were screened based on a qualitative score, with scores <36 considered inaccurate and excluded; for GC-MS data, a score threshold of 70 was applied and scores <70 were excluded. Internal

standards, noise peaks, column bleed peaks and derivatization reagent peaks were also removed.

For normalization, GC-MS data were normalized using internal standard-based segmental normalization, where each sample was normalized using internal standards with relative standard deviation <0.1. Metabolite identification confidence was assigned according to the Metabolomics Standards Initiative (MSI) guidelines (13). For LC-MS/MS, Level 1 was defined as retention time $\pm$ 0.3 min and fragmentation score $\geq$ 45, Level 2 as retention time $\pm$ 0.3 min and fragmentation score <45, Level 3 as fragmentation score $\geq$ 45 and Level 4 as fragmentation score <45. For GC-MS, Level 1 was defined as score $\geq$ 85, Level 2 as $\leq$ 80 score <85 and Level 3 as 70 $\leq$ score <80. In the present exploratory untargeted study, level confidence filtering was defined as the retention of all identified metabolites with MSI confidence Levels 1-4, as exclusion of lower-confidence metabolites may lead to loss of biologically relevant signals. When the same metabolite was identified by both platforms, the metabolite intensity from the platform with the higher qualitative score was retained. After stringent filtering, the high-confidence metabolite matrices from LC-MS/MS (positive and negative ion modes) and GC-MS were kept separately. Multivariate statistical analyses [including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA)] and differential metabolite screening were performed independently on the dataset of each platform. The lists of differential metabolites identified from both platforms were then integrated for subsequent metabolic pathway enrichment analysis.

Multivariate statistical analysis. Multivariate statistical analyses were performed independently on the LC-MS/MS and GC-MS datasets. PCA was performed to visualize overall distribution and assess analytical stability. PLS-DA and OPLS-DA were applied to discriminate metabolic profiles between groups and identify differential metabolites on each platform separately. Variable importance in projection (VIP) values were calculated from the OPLS-DA model. Fold change (FC) was calculated as the ratio of mean metabolite abundance in GSRCC vs. control groups. False discovery rate (FDR) correction (q-value) was applied using the Benjamini-Hochberg method. Metabolites with VIP >1, $q < 0.05$, and $\log_2$ FC >0.58 or $\log_2$ FC <-0.58 were considered significantly different between groups. OPLS-DA model robustness was evaluated using 7-fold cross-validation and 200-fold response permutation testing. A Q^2 intercept <0 determined the absence of overfitting.

Metabolic pathway enrichment analysis. Differential metabolites were mapped to metabolic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.kegg.jp/>). Pathway enrichment analysis was performed using hypergeometric testing to identify significantly perturbed pathways ($P < 0.05$, $q < 0.05$). Gene set enrichment analysis was conducted to visualize activated and suppressed pathways.

Clinical biochemistry analysis. Routine clinical biochemistry parameters, including cholinesterase, total protein, albumin and hemoglobin were measured in both discovery and validation

Table I. Clinical characteristics of the discovery cohort.

Characteristic	Healthy control (n=10)	GSRCC (n=8)	P-value
Sex, n (%)			
Male	5 (50.0)	3 (37.5)	
Female	5 (50.0)	5 (62.5)	
BMI	22.90 (21.93-24.63)	21.15 (20.02-26.68)	0.594
TNM stage, n (%)			
I-II	Not applicable	4 (50.0)	
III-IV	Not applicable	4 (50.0)	
Lymph node metastasis, n (%)			
Yes	Not applicable	5 (62.5)	
No	Not applicable	3 (37.5)	

Data are presented as the median (interquartile range) for BMI. $P < 0.05$ was considered to indicate a statistically significant difference. GSRCC, gastric signet-ring cell carcinoma.

Table II. Clinical characteristics of the validation cohort.

Characteristic	Healthy control (n=35)	GSRCC (n=35)	P-value
Sex, n (%)			
Male	18 (51.4)	17 (48.6)	
Female	17 (48.6)	18 (51.4)	
BMI	23.17 $\pm$ 1.54	24.00 $\pm$ 3.01	0.150
TNM stage, n (%)			
I-II	Not applicable	19 (54.3)	
III-IV	Not applicable	16 (45.7)	
Lymph node metastasis, n (%)			
Yes	Not applicable	27 (77.1)	
No	Not applicable	8 (22.9)	

Data are presented as the mean $\pm$ SD for BMI. $P < 0.05$ was considered to indicate a statistically significant difference. GSRCC, gastric signet-ring cell carcinoma.

cohorts using standard automated methods according to manufacturer's instructions. Cholinesterase was measured using reagents from Anhui Yipunokang Biotechnology Co., Ltd., total protein and albumin were measured using reagents from Shenzhen Mindray Bio-Medical Electronics Co., Ltd., and hemoglobin was measured using Sysmex Corporation reagents. BMI and TNM stage were considered to be potential confounders in result interpretation.

Statistical analysis. Benjamini-Hochberg method was applied to control for FDR due to multiple comparisons inherent in metabolomics data. Normality was assessed using the Shapiro-Wilk test. Continuous variables are expressed as the mean $\pm$ SD or median (interquartile range) as appropriate. Comparisons between groups were performed using unpaired Student's t-test for normally distributed data or Wilcoxon rank-sum test for non-normally distributed data. Categorical variables were compared using χ^2 test or Fisher's exact test. Receiver operating characteristic (ROC) curve analysis was

performed to evaluate diagnostic performance of candidate metabolites. Statistical analyses were conducted using SPSS (version 26.0; IBM Corp.) and GraphPad Prism (version 8.0; Dotmatics). $P < 0.05$ or $q < 0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of study participants. Summaries of clinical characteristics of the discovery and validation cohorts are shown in Tables I and II. In the discovery cohort, female patients predominated (62.5%), consistent with epidemiological patterns of this subtype. Nutritional status was assessed using body mass index (BMI), a widely used anthropometric indicator that reflects the balance between body weight and height and correlates with both undernutrition (BMI < 18.5) and overweight/obesity (BMI ≥ 25) in clinical studies. BMI exhibited no significant difference between groups ($P = 0.594$). TNM stage was classified using the American Joint Committee

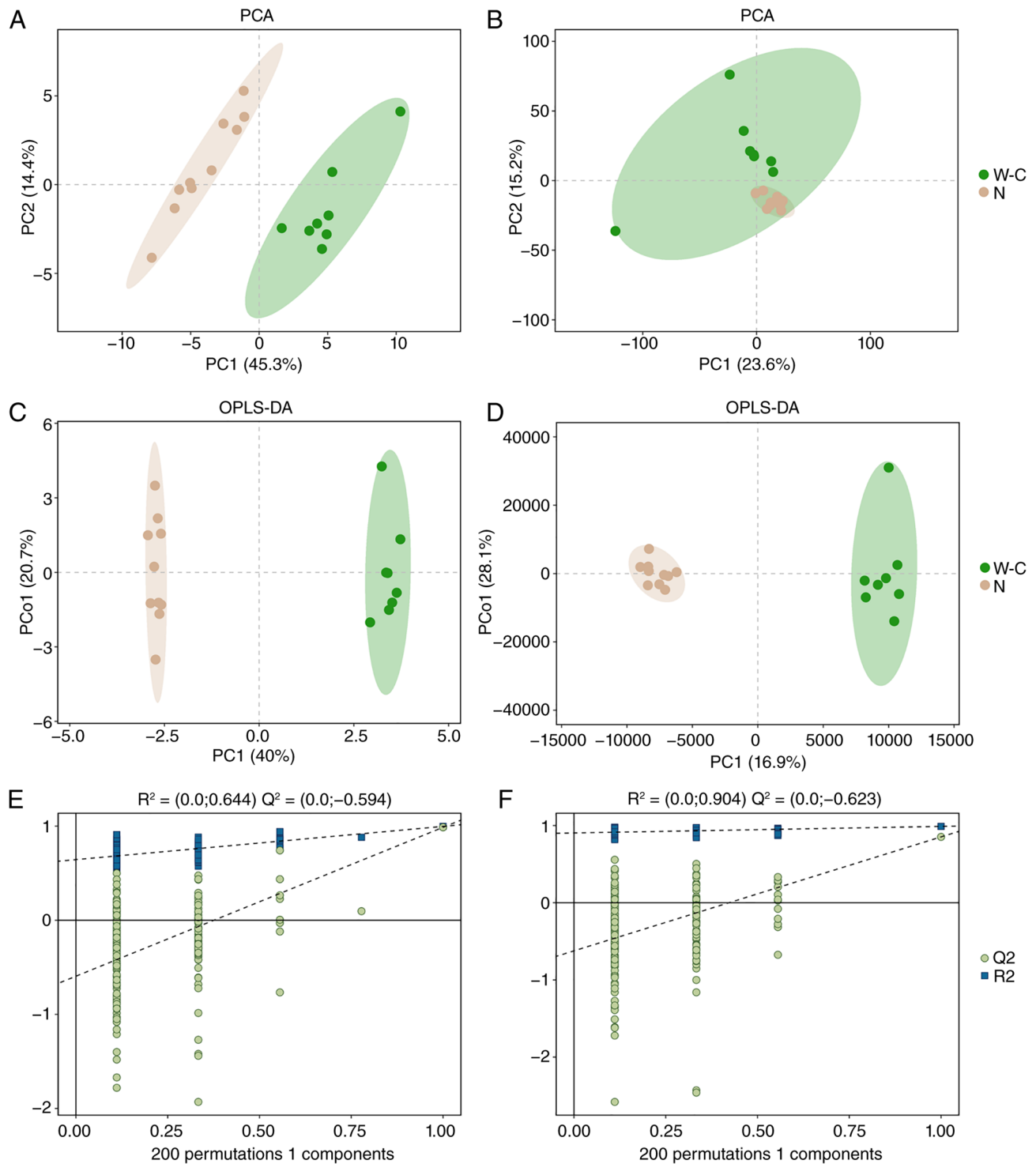


Figure 1. Multivariate analysis of GC-MS and LC-MS data. (A, C and E) GC-MS and (B, D and F) LC-MS. (A and B) PCA score plots (variance explained on axes). (C and D) OPLS-DA score plots. (E and F) Permutation tests (200 permutations), demonstrating model validity and absence of overfitting. W-C, GSRCC group; N, healthy control; GC-MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; OPLS-DA, orthogonal partial least squares discriminant analysis.

on Cancer 8th edition system (14), with 50.0% being stage I-II and 50.0% being stage III-IV. Lymph node metastasis was present in 5 patients (62.5%). In the validation cohort, sex and BMI were found to be well balanced ($P=0.150$). TNM staging distribution was as follows: Stage I-II in 19 patients (54.3%) and stage III-IV in 16 patients (45.7%). Lymph node metastasis was present in 27 patients (77.1%). These variables were

evaluated descriptively as potential confounders rather than statistically adjusted.

Multivariate statistical analysis. PCA score plots demonstrated clear separation between GSRCC (W-C) and healthy control groups (N), indicating distinct metabolic phenotypes (Fig. 1A and B). Supervised OPLS-DA models

Table III. Differential metabolites between patients with gastric signet-ring cell carcinoma and healthy controls.

No.	Metabolite	m/z	VIP	P-value	q-value	log ₂ FC	Trend
1	PC(P-18:0/20:4(6E,8Z,11Z,14Z)-OH(5S))	810.60	23.98	0.0010	0.0204	-0.79	Down
2	PE(19:0/18:0)	761.59	18.60	<0.0001	0.0034	-0.74	Down
3	PE(19:0/22:2(13Z,16Z))	813.62	8.75	0.0036	0.0431	-0.59	Down
4	PC(20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z))	832.58	8.09	0.0025	0.0354	-0.60	Down
5	Testosterone sulfate	367.16	6.74	0.0010	0.0204	-1.37	Down
6	Phenylalanylphenylalanine	313.15	6.47	0.0014	0.0247	-1.12	Down
7	DG(15:0/0:0/18:1(9Z)-O(12,13))	617.48	5.99	0.0041	0.0477	-0.88	Down
8	9-elaïdic acid	281.25	5.59	0.0016	0.0267	1.74	Up
9	PE(0:0/16:0)	454.29	4.06	0.0035	0.0428	0.76	Up
10	9S,10R-Epoxy-6Z-octadecene	282.28	4.03	0.0008	0.0184	-2.32	Down
11	PC(20:4(5Z,8Z,11Z,14Z)-OH(20)/P-18:0)	854.59	3.72	0.0002	0.0073	-1.13	Down
12	1-(2-Hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole	188.07	3.13	0.0029	0.0385	1.68	Up
13	3,5-Dihydroxytetradecanoylcarnitine	805.58	3.04	0.0000	0.0021	-1.34	Down
14	1,5-Naphthalenediamine	159.09	2.83	0.0034	0.0417	-2.12	Down
15	PE(0:0/20:4(5Z,8Z,11Z,14Z))	500.28	2.83	0.0018	0.0278	0.74	Up
16	Cholest-5-ene	388.39	2.60	<0.0001	0.0002	-1.09	Down
17	Dihydroxyacetone phosphate acyl ester	197.99	2.35	0.0034	0.0422	-1.36	Down
18	Octadeca-11E,13E,15Z-trienoic acid	296.26	2.28	0.0010	0.0203	-2.73	Down
19	Palmitoleoyl-EA	298.27	2.25	0.0007	0.0164	-2.81	Down
20	Farnesyl acetone	280.26	2.22	0.0001	0.0058	-3.75	Down

P<0.05 and q<0.05 were considered to indicate a statistically significant difference. VIP, variable importance in projection; FC, fold change; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DG, diacylglycerol; Down, downregulated; Up, upregulated.

further enhanced group discrimination (Fig. 1C and D), with permutation testing ($Q^2=-0.594$, GC-MS and $Q^2=-0.623$, LC-MS, both <0) demonstrating model validity and absence of overfitting (Fig. 1E and F). These results collectively demonstrated significant metabolic alterations in patients with GSRCC.

Identification of differential metabolites. Using criteria of VIP >1, $q<0.05$ and $\log_2 FC >0.58$, 68 differential metabolites were identified between patients with GSRCC and healthy controls, comprising 25 upregulated and 43 downregulated metabolites. The top 20 differential metabolites are presented in Table III, predominantly comprising phospholipids [PC and phosphatidylethanolamine (PE)] and amino acids. However, after FDR correction, no differential metabolites were identified between patients with GSRCC vs. without lymph node metastasis, nor between stage III-IV and stage I-II patients ($q>0.05$). Volcano plots and hierarchical clustering heatmaps visually summarize the differential metabolic patterns between groups (Fig. 2A and B).

Metabolic pathway enrichment analysis. KEGG pathway enrichment analysis identified 93 mapped pathways, with four pathways meeting significance criteria ($P<0.05$, $q<0.05$): 'Choline metabolism in cancer', 'glycerophospholipid metabolism', 'pentose and glucuronate interconversions' and 'arginine biosynthesis'. The top 10 enriched pathways are presented in Table IV and presented as bar and

bubble plots in Fig. 2C and D. Choline metabolism and glycerophospholipid metabolism emerged as the most prominently perturbed pathways, with their associated metabolites, PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] and dihydroxyacetone phosphate (DHAP) acyl ester showing marked alterations (Fig. 3A and B). ROC curve analysis demonstrated the diagnostic performance of these metabolites. PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] exhibited an area under the curve (AUC) of 0.89 and DHAP acyl ester 0.99 (Fig. 3C and D). It should be noted that ROC estimates derived from small discovery cohorts may overestimate diagnostic performance.

Clinical biochemistry parameters. To complement the metabolomic findings and assess systemic metabolic status in GSRCC, routine clinical biochemistry parameters were measured in both discovery and validation cohorts (Table V). In both cohorts, patients with GSRCC demonstrated significantly lower levels of cholinesterase, total protein, albumin and hemoglobin compared with healthy controls ($P<0.01$). However, no significant differences were observed between stage I-II and stage III-IV patients for any of these parameters ($P>0.05$), suggesting that these abnormalities were not closely associated with tumor stage progression. These findings indicate notable protein metabolic dysregulation and compromised nutritional status in patients with GSRCC, which aligns with the broader metabolic reprogramming suggested by pathway enrichment.

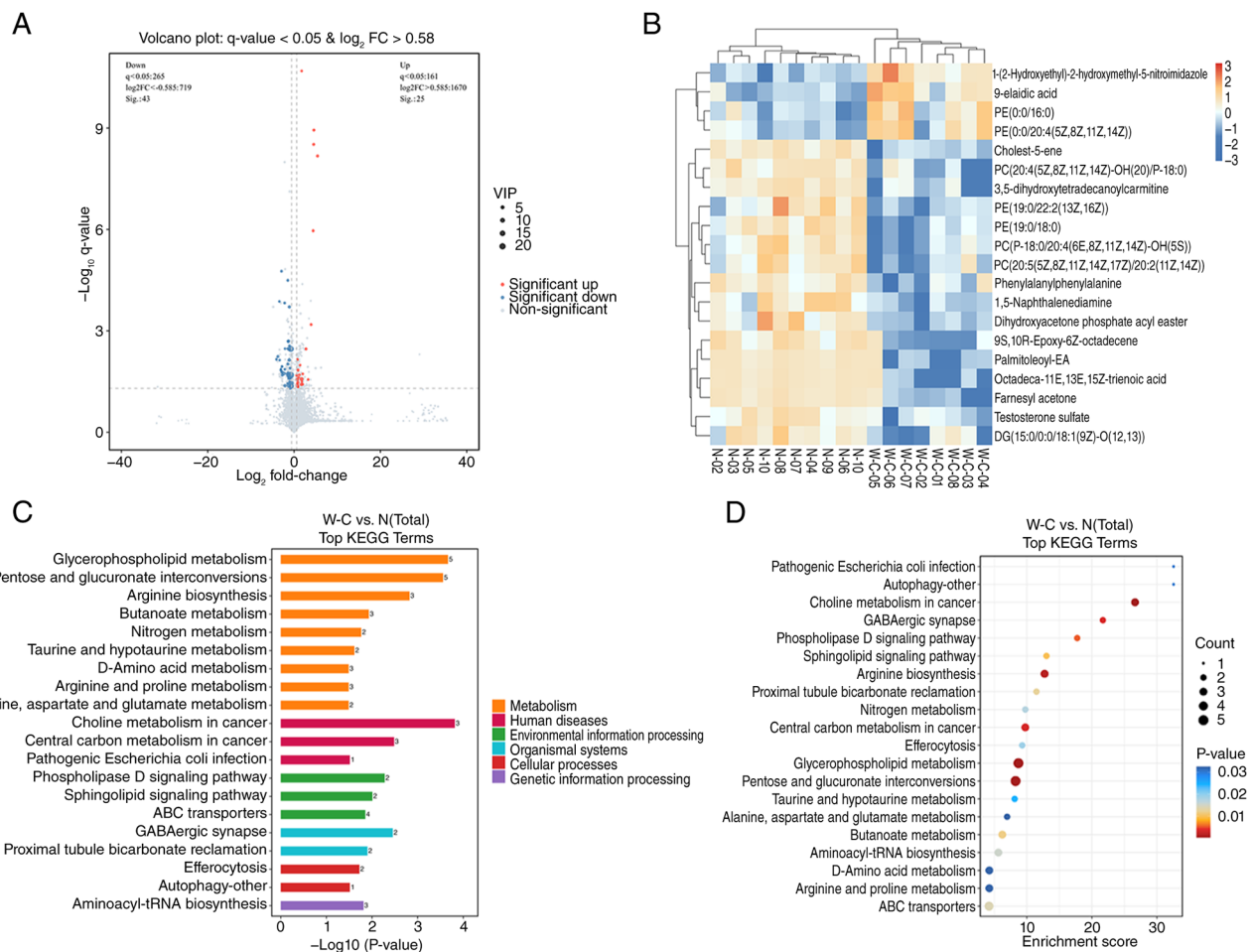


Figure 2. Differential metabolites and KEGG pathway enrichment. (A) Volcano plot of differential metabolites. Red represents upregulated genes (25), blue represented downregulated genes (43) and gray represents non-significant genes. Dot size represents VIP value, with larger dots indicating higher VIP scores. (B) Heatmap of selected differential metabolites. Red indicates a high abundance and blue indicates a low abundance. (C) Bar plot and (D) bubble plot of top enriched KEGG pathways. Color gradient indicates P-value (darker colors indicate smaller P-values). The size of the bars (C) or the bubbles (D) represents metabolite count. VIP, variable importance in projection; W-C, GSRCC group; N, healthy control; KEGG, Kyoto Encyclopedia of Genes and Genomes; ABC, ATP-binding cassette; FC, fold change.

Discussion

GSRCC represents a biologically aggressive subtype of gastric adenocarcinoma characterized by diffuse infiltration, poor differentiation and high metastatic potential, frequently presenting at advanced stages with unfavorable outcomes (15). The emergence of metabolomics has provided unprecedented opportunities to elucidate cancer-associated metabolic reprogramming and identify novel diagnostic biomarkers (16,17). In the present study, a comprehensive dual-platform metabolomics approach integrating LC-MS and GC-MS was employed to characterize the serum metabolomic landscape of GSRCC, an approach that offers broader metabolomic coverage compared with single-platform analyses commonly reported in gastric cancer metabolomics literature (18,19).

The present analysis identified 68 differential metabolites in patients with GSRCC, predominantly comprising phospholipids (PC and PE) and amino acids. Pathway enrichment analysis revealed notable perturbations in 'choline metabolism in cancer', 'glycerophospholipid metabolism', 'pentose and glucuronate interconversions' and 'arginine biosynthesis'. Notably, choline metabolism and glycerophospholipid

metabolism emerged as the most prominently dysregulated pathways, consistent with growing evidence implicating lipid metabolic reprogramming in gastric carcinogenesis (20,21). The underlying biological unit and integrated functional context of the dysregulated metabolic system were further emphasized. The perturbed metabolites were not independent events but were functionally interconnected within core biological modules, including choline metabolism, glycerophospholipid metabolism, membrane turnover, energy homeostasis and mitochondrial function, which collectively may contribute to the malignant progression of GSRCC. Considering these interconnected biological units strengthens the biological importance and contributions of the present study.

Choline metabolism serves multifaceted roles in cancer biology. As a precursor for acetylcholine neurotransmitter synthesis, choline also participates in ketone body and fatty acid metabolism via its metabolite betaine, which links methyl group metabolism to mitochondrial function by providing methyl donors for carnitine biosynthesis, thereby facilitating long-chain fatty acid transport into mitochondria for β -oxidation and subsequent acetyl-CoA production for

Table IV. Significantly enriched metabolic pathways in GSRCC.

ID	Pathway name	Key metabolites	P-value	q-value
hsa05231	Choline metabolism in cancer	Choline, PC(16:0/0:0) and PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)]	0.0002	0.0086
hsa00564	Glycerophospholipid metabolism	Choline, PE[16:0/20:2(11Z,14Z)], DHAP, PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] and PC(16:0/0:0)	0.0002	0.0086
hsa00040	Pentose and glucuronate interconversions	Oxoglutaric acid, glycerol, adonitol, L-arabitol and 2,3-Diketo-L-gulonate	0.0003	0.0086
hsa00220	Arginine biosynthesis	L-glutamic acid, oxoglutaric acid and citrulline	0.0015	0.0350
hsa05230	Central carbon metabolism in cancer	L-glutamic acid, oxoglutaric acid and L-leucine	0.0033	0.0542
hsa04727	GABAergic synapse	L-glutamic acid and oxoglutaric acid	0.0035	0.0542
hsa04072	Phospholipase D signaling pathway	L-glutamic acid and sphingosine-1-phosphate	0.0053	0.0701
hsa04071	Sphingolipid signaling pathway	SM[d18:1/24:1(15Z)] and sphingosine-1-phosphate	0.0098	0.1141
hsa00650	Butanoate metabolism	L-glutamic acid, oxoglutaric acid and 3R-hydroxy-butanoic acid	0.0117	0.1168
hsa04964	Proximal tubule bicarbonate reclamation	L-glutamic acid and oxoglutaric acid	0.0126	0.1168

Key metabolites listed are based on pathway databases; only PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] and DHAP acyl ester were significant after FDR correction ($q < 0.05$). GSRCC, gastric signet-ring cell carcinoma; DHAP, dihydroxyacetone phosphate; FDR, false discovery rate; GABA; gamma-aminobutyric acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin.

ketogenesis (22). Choline is a component of cellular and mitochondrial membranes and regulates lipid and energy homeostasis (23). Phosphatidylcholine and phosphatidylethanolamine, the major phospholipid classes affected in the present study, collectively account for ~50% of cellular membrane phospholipids (24). The observed downregulation of PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] may reflect altered fatty acid composition in malignant cell membranes associated with poor differentiation and aggressive behavior. This PUFA-rich PC species is critical for maintaining optimal membrane fluidity and lipid raft integrity. Its downregulation may impair membrane biophysical properties, leading to: i) Enhanced lateral mobility and clustering of receptor tyrosine kinases, promoting sustained MAPK and PI3K/Akt activation (25); ii) disrupted Ras nanodomain organization, facilitating downstream oncogenic signaling via flotillin-1-mediated Ras-SOS1 interactions (26); and iii) altered membrane curvature, which enhances tumor cell migration and invasion (27). Collectively, these changes create a permissive environment for cytoskeletal remodeling, ultimately contributing to poor differentiation and dissemination in GSRCC.

The association between choline metabolism and metastatic potential has been previously recognized (28). GSRCC exhibits a unique immunosuppressive tumor microenvironment that facilitates peritoneal dissemination, with mucinous components infiltrating surrounding stroma and promoting tumor cell spread to the peritoneal cavity (29,30). Mechanistically, choline and glycerophospholipid metabolites may influence tumor

progression through modulation of MAPK, PI3K/Akt/mTOR and Ras signaling pathways, which are well-established drivers of gastric cancer proliferation and metastasis (31,32). Specifically, altered PC/PE ratios remodel membrane fluidity and charge, affecting Ras nanoclustering and Raf-dependent MAPK activation (31). Meanwhile, glycerophospholipid turnover generates PA and LPA, which directly activate mTORC1 and PI3K/Akt via allosteric binding and receptor-mediated mechanisms (32). PI3K/Akt pathway activation has been specifically associated with lymph node metastasis and poor prognosis in gastric cancer (33). Despite this, these hypotheses require further experimental determination.

Notably, the marked downregulation of DHAP acyl ester in GSRCC serum, with an AUC of 0.99, suggested superior diagnostic potential. DHAP acyl ester serves as a key intermediate in lipid metabolism, participating in glycerophospholipid and glycerolipid biosynthesis (34). A number of mechanisms may account for its reduced circulating levels in GSRCC. Enhanced utilization of DHAP acyl ester as a precursor for membrane phospholipid synthesis in rapidly proliferating tumor cells could deplete circulating pools. In addition, the Warburg effect, characterized by aerobic glycolysis in cancer cells, may redirect DHAP flux toward glycolytic pathways rather than lipid synthesis (35). Impaired mitochondrial function in gastric cancer cells may further compromise normal lipid metabolism, reducing DHAP acyl ester production. These findings warrant further investigation into the precise regulatory mechanisms governing DHAP acyl ester metabolism in gastric cancer and its potential interplay with glucose and amino acid metabolism.

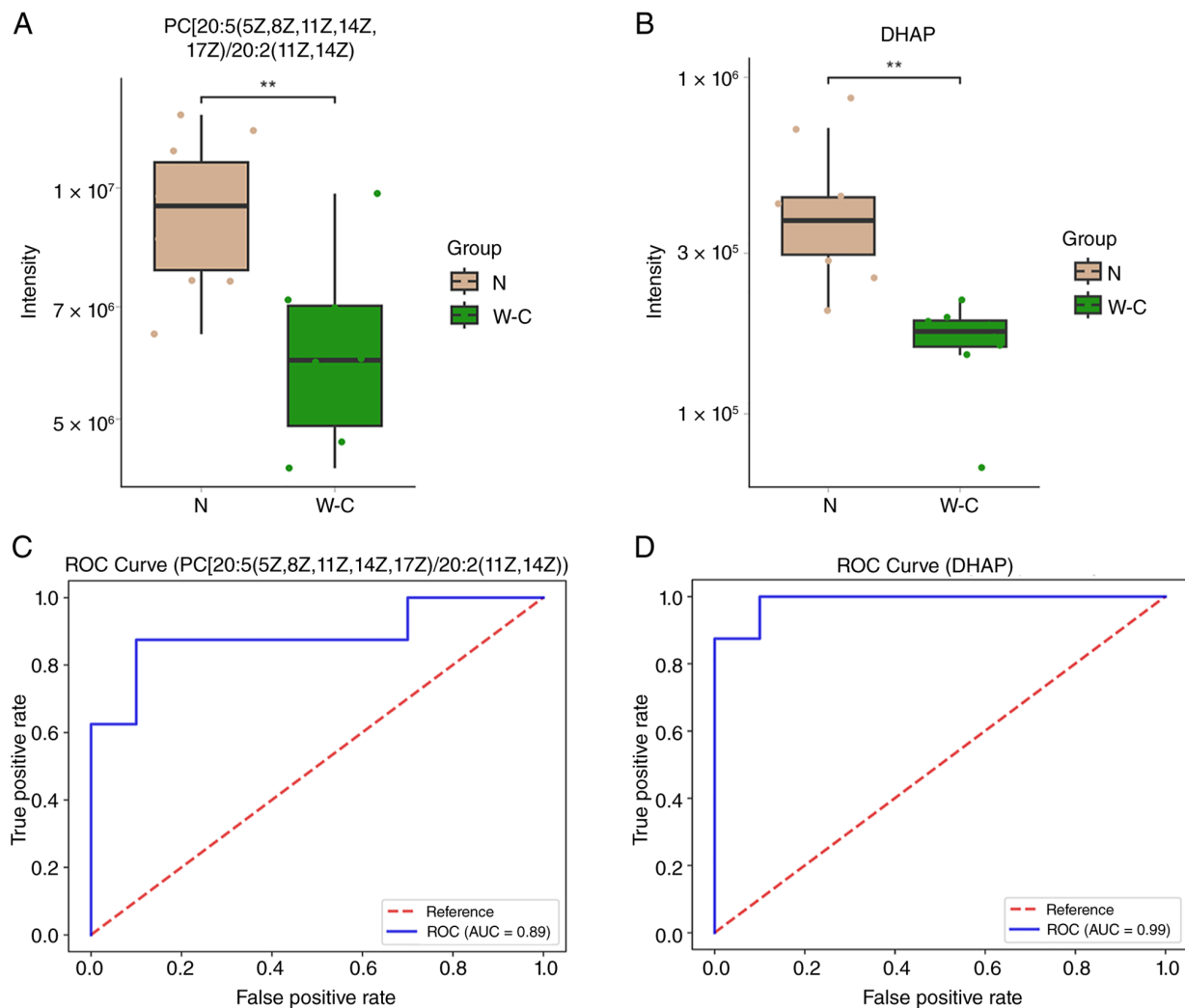


Figure 3. Differential abundance and diagnostic performance of key metabolites. Box plots of (A) PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] and (B) DHAP acyl ester in patients with GSRCC and healthy controls. Data are presented as the mean $\pm$ SD. $P < 0.05$ vs. healthy control. ROC curves for (C) PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] and (D) DHAP acyl ester. The AUC values are 0.89 and 0.99, respectively. ** $P < 0.01$. W-C, GSRCC group; N, healthy control; ROC, receiver operating characteristic; AUC, area under the curve; DHAP, dihydroxyacetone phosphate.

To complement the metabolomic findings within conventional clinical parameters, routine biochemistry markers related to choline metabolism and nutritional status were assessed. The marked reductions in cholinesterase, total protein, albumin and hemoglobin in patients with GSRCC across both discovery and validation cohorts provide complementary evidence of systemic metabolic dysregulation. Cholinesterase, synthesized primarily in the liver, serves as a marker of hepatic synthetic capacity; its decline in patients with GSRCC suggests impaired liver function secondary to cancer-associated cachexia or metabolic stress. The concordant reductions in protein markers indicate profound protein metabolic disturbances and compromised nutritional status, consistent with the cachexia syndrome frequently accompanying advanced malignancy (36). However, BMI did not notably differ between patients with GSRCC and controls, and no notable differences were observed across TNM stages. The present findings suggest that GSRCC-associated metabolic disturbances occur early in tumorigenesis, leading to preferential depletion of protein reserves through systemic inflammation and amino acid catabolism, with relative preservation of adipose tissue. When interpreted alongside the pathway enrichment findings (choline

and glycerophospholipid metabolism), these conventional parameters offer complementary information on the systemic metabolic status of patients with GSRCC.

A number of limitations of the present study warrant consideration. First, the modest sample size, particularly in the discovery cohort, reflects the relative rarity of GSRCC and the exploratory nature of the present investigation. Although strict model validation was applied to avoid overfitting, the findings should be regarded as exploratory rather than confirmatory. The lack of disease control groups (namely, non-signet-ring gastric adenocarcinoma and benign gastric diseases) prevents determination of whether the observed metabolic signatures are specific to GSRCC or reflect broader cancer-associated alterations. Third, the present study performed non-targeted profiling without subsequent targeted LC-MS/MS validation; therefore, candidate metabolites should be considered exploratory signatures rather than fully determined biomarkers. Fourth, functional validation is required to elucidate mechanistic roles. Future studies with larger cohorts, well-matched disease controls, targeted metabolomics and experimental validation are needed to validate specificity, robustness and clinical applicability.

Table V. Clinical biochemistry parameters in discovery and validation cohorts.

Parameter	Cohort	Healthy control	Total GSRCC	TNM stage I-II	TNM stage III-IV	T vs. N	P-value		
							Stage I-II vs. N	Stage III-IV vs. N	Stage I-II vs. III-IV
Cholinesterase, U/l	Discovery	297.80±73.59	200.63±48.98	203.25±41.10	198.00±62.37	0.006	0.034	0.035	0.893
	Validation	317.49±61.20	246.63±60.23	236.89±45.95	258.19±73.66	<0.001	<0.001	0.004	0.304
Total protein, g/l	Discovery	71.75±4.02	60.04±7.73	55.93±6.68	64.17±7.03	0.001	<0.001	0.024	0.140
	Validation	69.87±4.67	62.83±5.70	63.35±5.21	62.21±6.34	<0.001	<0.001	<0.001	0.564
Albumin, g/l	Discovery	45.88±1.97	37.00±3.71	35.36±3.59	38.63±3.49	<0.001	<0.001	<0.001	0.242
	Validation	44.37±2.54	39.05±3.48	38.77±2.73	39.38±4.27	<0.001	<0.001	<0.001	0.614
Hemoglobin, g/l	Discovery	142.00	119.50	112.00±16.99	115.25±14.50	0.007	0.013	0.021	0.781
	Validation	(124.75-149.50)	(99.50-124.00)	128.68±22.37	132.00 (110.50-139.50)	<0.001	0.006	0.007	0.855

Data are presented as the mean ± SD or median (interquartile range) as appropriate. P<0.05 was considered to indicate a statistically significant difference. GSRCC, gastric signet-ring cell carcinoma; N, healthy control; T, total GSRCC.

In conclusion, the present study provides, to the best of our knowledge, the first comprehensive metabolomic characterization of GSRCC using a dual-platform mass spectrometry approach. Notable perturbations in choline and glycerophospholipid metabolism were identified, with PC[20:5(5Z,8Z,11Z,14Z,17Z)/20:2(11Z,14Z)] and DHAP acyl ester emerging as exploratory candidate metabolic signatures associated with GSRCC, which may establish a foundation for future biomarker discovery and contribute to the evolving understanding of GSRCC biology.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81703830), the Natural Science Foundation of Jiangsu Province (grant no. BK20241146), the Jiangsu Provincial Traditional Chinese Medicine Science and Technology Development Program (grant no. MS2025016) and the Affiliated Hospital of Nanjing University of Chinese Medicine Program (grant no. Y24042).

Availability of data and materials

The data generated in the present study may be found in the OMIX database of the National Genomics Data Center under accession number (OMIX017401) or at the following URL: (persistent, <https://ngdc.cncb.ac.cn/omix/release/OMIX017401>).

Authors' contributions

HC, SG, JL and WG contributed to the present study conception and design. HC, YB, JS, JW, FM, WS, QS and WG contributed to material preparation, data collection and analysis. HC, SG and JL wrote the first draft of the manuscript and all authors commented on previous versions. All authors read and approved the final version of the manuscript. HC, SG and WG confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the ethics committee of Jiangsu Province Hospital of Chinese Medicine (approval nos. 2021NL-187-02, 2021NL-187-03, 2021NL-187-04 and 2021NL-187-05). This ethics approval was issued for a broader clinical cohort study, and the present metabolomics study was conducted within the scope of this approved protocol. The ethics approval has been extended to cover the entire study period, including the serum sample collection period. Written informed consent was obtained from each participant prior to enrollment and their consent for the storage of serum samples for future secondary analyses was also obtained.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

References

- Chen Y, Wang B, Zhao Y, Shao J, Shen X, Zhang L, Gu W, Xu J, Jiang J, Xu L, *et al*: Metabolomic machine learning predictor for diagnosis and prognosis of gastric cancer. *Nat Commun* 15: 1657, 2024.
- Yang WJ, Zhao HP, Yu Y, Wang JH, Guo L, Liu JY, Pu J and Lv J: Updates on global epidemiology, risk and prognostic factors of gastric cancer. *World J Gastroenterol* 29: 2452-2468, 2023.
- Zhao W, Jia Y, Sun G, Yang H, Liu L, Qi X, Ding J, Yu H, Xu B, Zhao S, *et al*: Single-cell analysis of gastric signet ring cell carcinoma reveals cytological and immune microenvironment features. *Nat Commun* 14: 2985, 2023.
- Kushima R: The updated who classification of digestive system tumours-gastric adenocarcinoma and dysplasia. *Pathology* 43: 8-15, 2022.
- Liang Z, Zheng L and Cao J: Endoscopic and pathological examinations of early-signet-ring carcinoma in the stomach. *Healthcare (Basel)* 13: 2689, 2025.
- Yan J, Liu Y, Liu T and Zhu Q: A predictive and prognostic model for metastasis risk and prognostic factors in gastrointestinal signet ring cell carcinoma. *Eur J Med Res* 29: 545, 2024.
- Tang Y, Ren L and Mao T: Update on diagnosis and treatment of early signet-ring cell gastric carcinoma: A literature review. *World J Gastrointest Endosc* 15: 240-247, 2023.
- Pappas A, Tan WK, Waldoock W, Richardson S, Tripathi M, Januszewicz W, Roberts G, O'Donovan M, Fitzgerald RC and di Pietro M: Single-bite versus double-bite technique for mapping biopsies during endoscopic surveillance for hereditary diffuse gastric cancer: A single-center, randomized trial. *Endoscopy* 53: 246-253, 2021.
- Shi Y, Yang Q, Xu B, Yuan C, Qi K, Pan Y, Wang Z, Chen Q, Sun D, Zhao W, *et al*: Key lipid reprogramming revealed in gastric signet ring cell carcinoma by spatial mass spectrometry metabolomics. *J Am Soc Mass Spectrom* 36: 1598-1608, 2025.
- Fu L, Li M, Lv J, Yang C, Zhang Z, Qin S, Li W, Wang X and Chen L: Deep neural network for discovering metabolism-related biomarkers for lung adenocarcinoma. *Front Endocrinol (Lausanne)* 14: 1270772, 2023.
- Sun D, Zhang Y, Wang D, Zhao X, Han R, Li N, Li X, Li T, Wang P, Jia Q, *et al*: Experimental study on changes in metabolic mechanism of papillary thyroid carcinoma complicated with hashimoto's thyroiditis. *Heliyon* 9: e20661, 2023.
- Wiedmann L, De Angelis Rigotti F, Vaquero-Siguero N, Donato E, Espinet E, Moll I, Alsina-Sanchis E, Bohnenberger H, Fernandez-Florio E, Mülthart R, *et al*: Hapln1 potentiates peritoneal metastasis in pancreatic cancer. *Nat Commun* 14: 2353, 2023.
- Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, Fan TW, Fiehn O, Goodacre R, Griffin JL, *et al*: Proposed minimum reporting standards for chemical analysis chemical analysis working group (CAWG) metabolomics standards initiative (MSI). *Metabolomics* 3: 211-221, 2007.
- Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR and Winchester DP: The eighth edition AJCC cancer staging manual: Continuing to build a bridge from a population-based to a more 'personalized' approach to cancer staging. *CA Cancer J Clin* 67: 93-99, 2017.
- Raju D, Prabhu S, Maria A, Shirodkar SNS, Damerla RR, Chakrabarty S, Shetty PS, Rao M and Kumar NAN: Gastric signet ring cell carcinoma: Tumor microenvironment reprogramming and novel therapeutic targets with emphasis on grin2d. *Clin Transl Sci* 18: e70424, 2025.
- Sun Y, Zhang X, Hang D, Lau HCH, Du J, Liu C, Xie M, Pan Y, Wang L, Liang C, *et al*: Integrative plasma and fecal metabolomics identify functional metabolites in adenoma-colorectal cancer progression and as early diagnostic biomarkers. *Cancer Cell* 42: 1386-1400.e8, 2024.
- Xiao Y, Ma D, Yang YS, Yang F, Ding JH, Gong Y, Jiang L, Ge LP, Wu SY, Yu Q, *et al*: Comprehensive metabolomics expands precision medicine for triple-negative breast cancer. *Cell Res* 32: 477-490, 2022.
- Matsuoka T and Yashiro M: Bioinformatics analysis and validation of potential markers associated with prediction and prognosis of gastric cancer. *Int J Mol Sci* 25: 5880, 2024.
- Wang F, Pang R, Zhao X, Zhou B, Tian Y, Ma Y and Rong L: Plasma metabolomics and lipidomics reveal potential novel biomarkers in early gastric cancer: An explorative study. *Int J Biol Markers* 39: 226-238, 2024.
- Xiao J, Cao S, Wang J, Li P, Cheng Q, Zhou X, Dong J, Li Y, Zhao X, Xu Z and Yang L: Leptin-mediated suppression of lipoprotein lipase cleavage enhances lipid uptake and facilitates lymph node metastasis in gastric cancer. *Cancer Commun (Lond)* 44: 855-878, 2024.
- Chen S, Chen W, Xu T, Li J, Yu J, He Y and Qiu S: The impact of aberrant lipid metabolism on the immune microenvironment of gastric cancer: A mini review. *Front Immunol* 16: 1639823, 2025.
- Chen X, Qiu W, Ma X, Ren L, Feng M, Hu S, Xue C and Chen R: Roles and mechanisms of choline metabolism in nonalcoholic fatty liver disease and cancers. *Front Biosci Landmark Ed* 29: 182, 2024.
- Yao N, Li W, Xu G, Duan N, Yu G and Qu J: Choline metabolism and its implications in cancer. *Front Oncol* 13: 1234887, 2023.
- Son Y, Kenny TC, Khan A, Birsoy K and Hite RK: Structural basis of lipid head group entry to the Kennedy pathway by flvcr1. *Nature* 629: 710-716, 2024.
- Mukerjee S, Saeedan AS, Ansari MN and Singh M: Polyunsaturated fatty acids mediated regulation of membrane biochemistry and tumor cell membrane integrity. *Membranes (Basel)* 11: 479, 2021.
- Jin H, Koh M, Lim H, Yong HY, Kim ES, Kim SY, Kim K, Jung J, Ryu WJ, Choi KY and Moon A: Lipid raft protein flotillin-1 is important for the interaction between SOS1 and H-Ras/K-Ras, leading to Ras activation. *Int J Cancer* 52: 1933-1946, 2023.
- Damalas A, Kyriazis ID, Tutkus M, Angelidis C and Trachana V: Membrane curvature and cancer: Mechanisms, implications, and therapeutic perspectives. *Cancers (Basel)* 18: 1076, 2026.
- Pan J, Zhang L, Wang X, Li L, Yang C, Wang Z, Su K, Hu X, Zhang Y, Ren G, *et al*: Chronic stress induces pulmonary epithelial cells to produce acetylcholine that remodels lung pre-metastatic niche of breast cancer by enhancing netosis. *J Exp Clin Cancer Res* 42: 255, 2023.
- Zhang J, Collingwood R, Al Diffalha S, Manna DD, Paluri RK, Mejbil HA and Gbolahan O: Comprehensive characterization of the immune microenvironment of colorectal and gastric signet ring cell cancer. *Cells* 15: 30, 2025.
- Xie Y, Li C, Yu M and Cao J: Immunosuppressive tumor micro-environment in gastric signet-ring cell carcinoma. *World J Clin Oncol* 15: 1126-1131, 2024.
- Zhou Y and Hancock JF: RAS nanoclusters are cell surface transducers that convert extracellular stimuli to intracellular signalling. *FEBS Lett* 597: 892-908, 2023.
- Wang C, Yang Z, Xu E, Shen X, Wang X, Li Z, Yu H, Chen K, Hu Q, Xia X, *et al*: Apolipoprotein C-II induces EMT to promote gastric cancer peritoneal metastasis via PI3K/AKT/mTOR pathway. *Clin Transl Med* 11: e522, 2021.
- Cao W, Zeng Z, Lan J, Yang Y, Lu M and Lei S: Knockdown of fut11 inhibits the progression of gastric cancer via the pi3k/akt pathway. *Heliyon* 9: e17600, 2023.
- Oh S, Jo S, Bajzikova M, Kim HS, Dao TTP, Rohlena J, Kim JM, Neuzil J and Park S: Non-bioenergetic roles of mitochondrial gpd2 promote tumor progression. *Theranostics* 13: 438-457, 2023.
- Singh J, Suh EH, Sharma G, Khemtong C, Sherry AD and Kovacs Z: Probing carbohydrate metabolism using hyperpolarized ¹³C-labeled molecules. *NMR Biomed* 32: e4018, 2019.
- Tamayo-Torres E, Garrido A, de Cabo R, Carretero J and Gómez-Cabrera MC: Molecular mechanisms of cancer cachexia. Role of exercise training. *Mol Aspects Med* 99: 101293, 2024.

