This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Detailed Methods

Sample preparation for metabolomics

Plasma samples were processed following previously reported method with some modifications. Briefly, 50 µL of plasma sample were mixed with 200 µL of ice-cold methanol and then the mixture was incubated for 30 min at 1500 rpm (4℃). The samples were further centrifuged for 10 min at 12,000 rpm (4℃) for protein precipitation. The supernatants were transferred to clean centrifuge tubes and evaporated using SpeedVac. Finally, the dried extracts were redissolved with 1% acetonitrile in water, and upper layer liquids were collected for metabolomic profiling.

Metabolomic profiling

Plasma metabolomic profiling was conducted using ultra high-performance liquid chromatography-mass spectrometry (LC-MS). Chromatographic separation was performed on ACQUITY UPLC HSS T3 columns (2.1 × 100 mm, 1.8 µm, Waters, Dublin, Ireland). Ultra-performance Liquid Chromatography (Agilent 1290 II, Agilent Technologies, Germany) coupled to Quadrupole-TOF MS (5600 Triple TOF Plus, AB Sciex, Singapore) was applied to acquire metabolome data. The electrospray ionization (ESI) source was set up in positive and negative ion modes, respectively. Mobile phase A was water containing 0.1% formic acid (v/v), and mobile phase B was acetonitrile containing 0.1% formic acid (v/v). The linear gradients were as follows: 1% B at 1 min, 40% B at 5 min, 50% B at 8 min, 65% B at 10 min, 70% B at 12 min, 100%B at 12.1. The temperature of the column was maintained at 40 °C, and

autosampler was set at 4 °C. The injected volume was 5 μL per run, and the flow rate was 0.35 mL/min.

The MS parameters for detection were: ESI, source voltage 5.5 kV or −4.5 kV; vaporizer temperature, 500 °C; drying gas (N2) pressure, 50 psi; nebulizer gas (N2) pressure, 50 psi; curtain gas (N2) pressure, 35 psi; The scan range was m/z 60-600. Information-dependent acquisition mode was used for MS/MS analyses of the metabolites. The collision energy was set at 35 ± 15 eV.

All detected ions were extracted using MarkerView 1.3 (AB Sciex, Concord, ON, Canada) into Excel in the format of two-dimensional matrix, including mass to charge ratio (m/z), retention time, and peak areas, and isotopic peaks were filtered. PeakView 2.2 (AB Sciex, Concord, ON, Canada) was applied to extract MS/MS data. Metabolites were identified by comparing their product ion spectra with standard references and open-source database, including HMDB (http://www.hmdb.ca/) and METLIN (https://metlin.scripps.edu/).

Quality control

Quality control (QC) samples were prepared using mixed plasma samples. At the beginning of each batch for LC-MS analysis, four consecutive QC samples were detected to balance analytical system, and then one QC sample was inserted at a regular interval of every ten injections. Ionization signals were monitored in QC samples based on the peak intensities of internal standards to ensure no drop-in intensity (within 20%) and no drift in retention time (within 0.05 min) throughout the run. In the present study, a total of 23 QC samples were included for all batches.
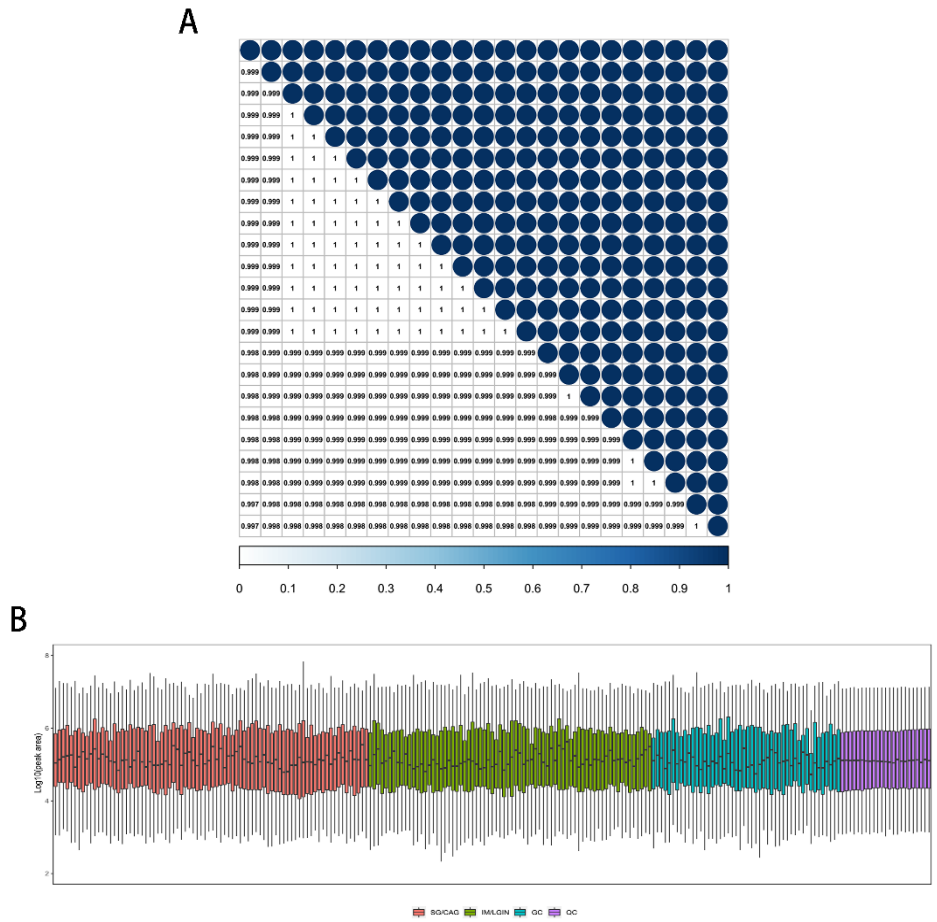
eFigure 1. Quality control during LC-MS analysis

(A) Spearman's correlation coefficients of 23 QC runs;

(B) Intensity distribution of metabolites for all study samples and QC runs.
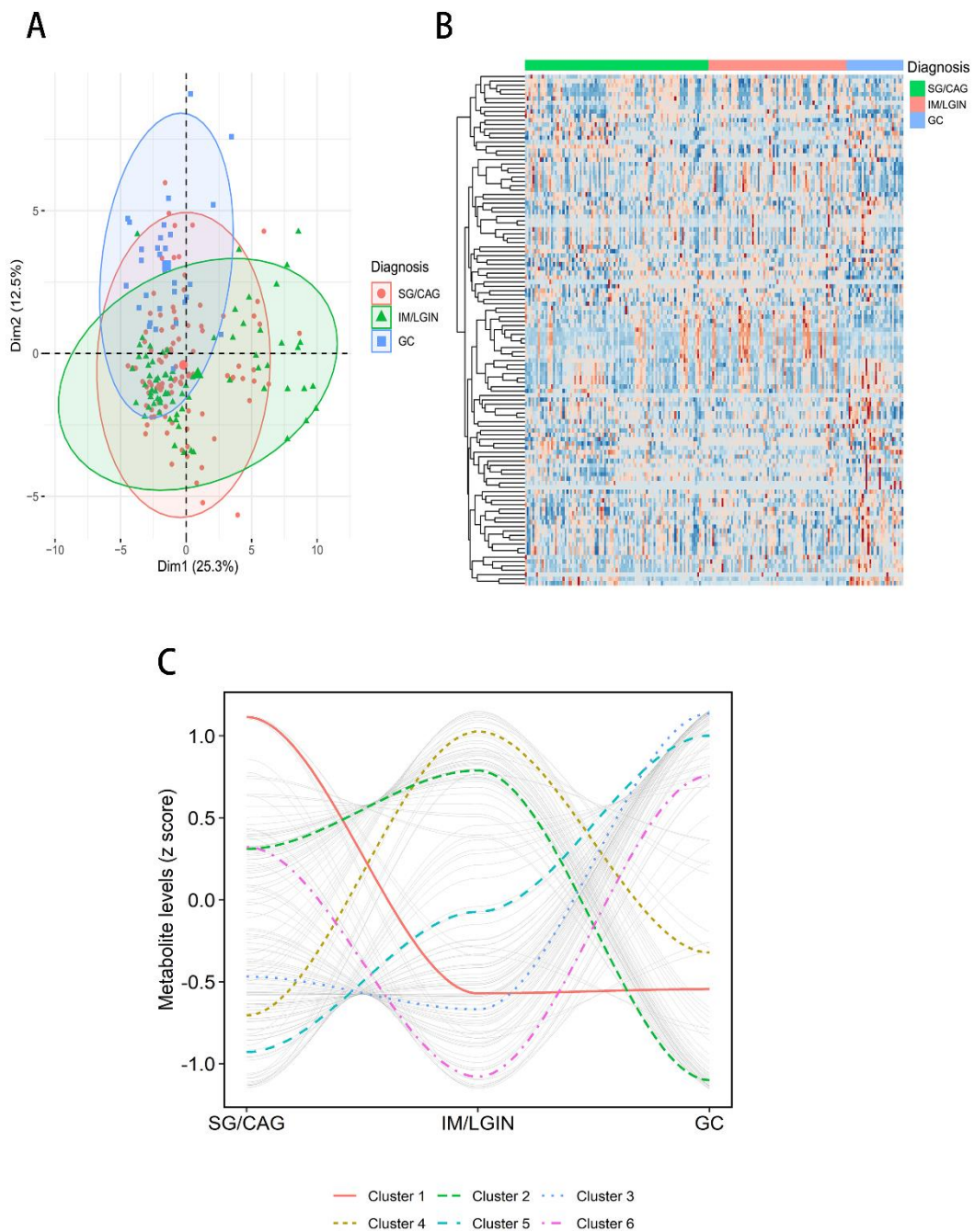
eFigure 2. Metabolomic profiles for precancerous gastric lesions and GC

(A) Score plot of PCA for metabolomic profiles in subjects with different gastric lesions and GC;

(B) Hierarchical clustering of metabolomic profiles in subjects with different gastric lesions and GC;

(C) Trajectories of metabolites from mild gastric lesions (SG or CAG) to advanced gastric lesions (IM or LGIN) and GC and the six identified clusters grouped by similar trajectories.

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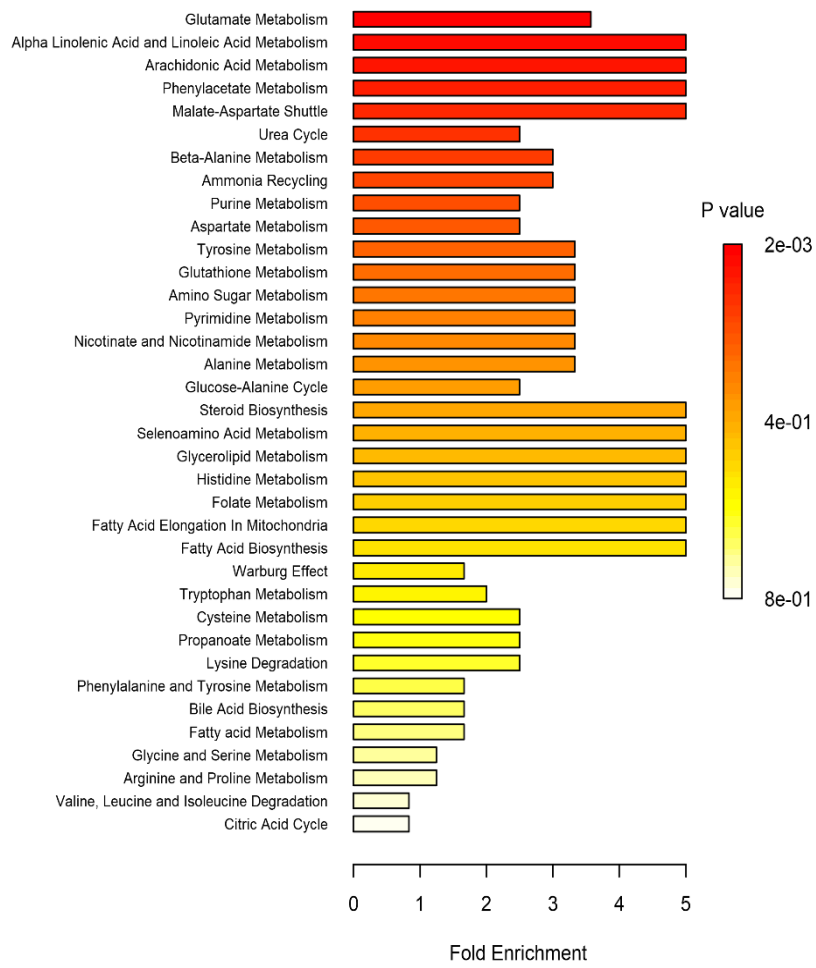
eFigure 3. Metabolite set enrichment analysis for the enriched metabolite pathways associated with GC.

Metabolite score		OR (95%CI)	P
Tertile 1		1.0 (Reference)	
Tertile 2		0.70 (0.22, 2.12)	0.53
Tertile 3		1.78 (1.04, 3.20)	0.04
Per uint increase of metabolite score		5.25 (1.01, 30.72)	0.05
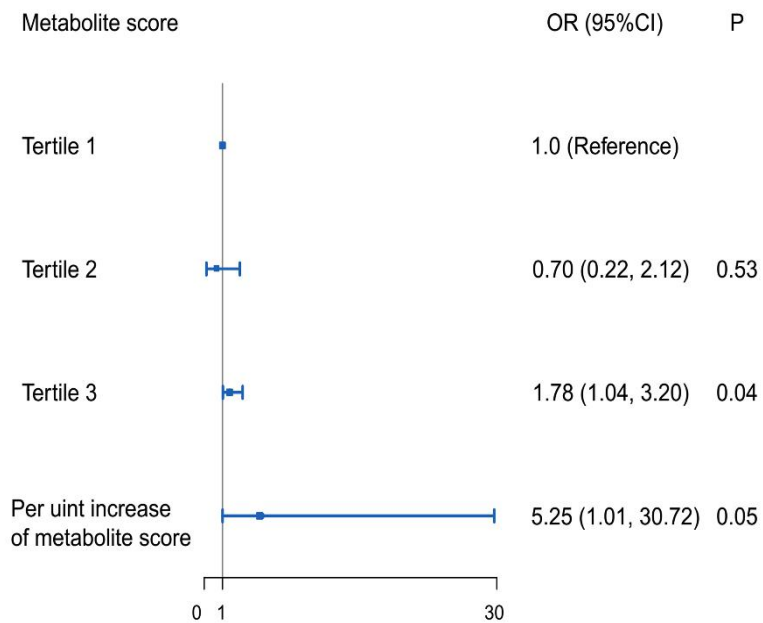
eFigure 4. Metabolite scores associated with the progression of gastric lesions during the follow-up.

The score was calculated based on three validated metabolites (alpha-linolenic acid, linoleic acid, and palmitic acid). Analyses were conducted by conditional logistic regression stratified on baseline gastric histopathology, with adjustment for age, sex, and *H.pylori* infection.

eTable 1. Characteristics of study subjects in the discovery and validation sets

Characteristics	Discovery set					Validation set			
	Total	SG/CAG	IM/LGIN	GC		Total	SG/CAG	IM/LGIN	GC
	(n = 200)	(n = 97)	(n = 73)	(n = 30)		(n = 200)	(n = 80)	(n = 72)	(n = 48)
Age (Mean ± SD)	56.8 ± 7.5	56.9 ± 7.4	55.7 ± 7.6	58.9 ± 7.6		57.5 ± 8.1	56.1 ± 8.5	57.2 ± 8.1	60.2 ± 6.8
Sex (%)									
Male	124 (62.0)	58 (59.8)	39 (53.4)	27 (90.0)		136 (68.0)	42 (52.5)	53 (73.6)	41 (85.4)
Female	76 (38.0)	39 (40.2)	34 (46.6)	3 (10.0)		64 (32.0)	38 (47.5)	19 (26.4)	7 (14.6)
H.pylori infection (%)									
Yes	98 (49.0)	43 (44.3)	43 (58.9)	12 (40.0)		107 (53.5)	40 (50.0)	39 (54.2)	28 (58.3)
No	102 (51.0)	54 (55.7)	30 (41.1)	18 (60.0)		93 (46.5)	40 (50.0)	33 (45.8)	20 (41.7)

CAG, chronic atrophic gastritis; LGIN, low-grade intraepithelial neoplasia; GC, gastric cancer; *H.pylori*, *Helicobacter pylori*; IM, intestinal metaplasia; SG, superficial gastritis

eTable 2. Plasma metabolites associated with advanced gastric lesions and gastric cancer[a]

Metabolites	Discovery set														Validation set								
	IM/LGIN *vs.* SG/CAG				GC *vs.* SG/CAG				Early GC[b] *vs.* SG/CAG					IM/LGIN *vs.* SG/CAG			GC *vs.* SG/CAG			Early GC[b] *vs.* SG/CAG		
	n/n	OR (95%CI)	*P*	FDR	n/n	OR (95%CI)	*P*	FDR	n/n	OR (95%CI)	*P*	FDR		n/n	OR (95%CI)	*P*	n/n	OR (95%CI)	*P*	n/n	OR (95%CI)	*P*
L-Aspartic acid	73/97	0.60 (0.39, 0.92)	.02	.29	30/97	2.68 (1.72, 4.37)	<.001	**.003**	22/97	2.43 (1.51, 4.10)	<.001	**.01**		72/80	0.84 (0.69, 2.12)	.17	48/80	0.68 (0.49, 0.96)	**.03**	31/80	0.87 (0.59, 1.27)	.27
sn-2 LysoPC(20:3)	73/97	1.31 (0.93, 1.87)	.12	.41	30/97	0.29 (0.14, 0.51)	<.001	**.003**	22/97	0.21 (0.08, 0.43)	<.001	**.01**		72/80	0.58 (1.19, 1.21)	.001	48/80	0.65 (0.45, 0.94)	**.03**	31/80	0.78 (0.53, 1.16)	.15
sn-1 LysoPC(18:3)	73/97	1.26 (0.90, 1.77)	.18	.47	30/97	0.27 (0.13, 0.50)	<.001	**.003**	22/97	0.26 (0.11, 0.51)	<.001	**.01**		72/80	0.68 (1.14, 1.26)	.02	48/80	0.62 (0.43, 0.88)	**.01**	31/80	0.76 (0.51, 1.11)	.12
p-Cresol sulfate	73/97	1.36 (0.99, 1.89)	.06	.41	30/97	3.56 (1.92, 7.49)	<.001	**.005**	22/97	3.32 (1.69, 7.50)	.001	**.02**		72/80	1.02 (0.27, 5.27)	.45	48/80	0.74 (0.53, 1.04)	.07	31/80	0.69 (0.47, 1.01)	.05
Uracil	73/97	0.81 (0.54, 1.21)	.31	.60	30/97	0.42 (0.25, 0.64)	<.001	**.005**	22/97	0.34 (0.19, 0.57)	<.001	**.01**		72/80	1.09 (0.44, 3.24)	.30	48/80	0.80 (0.58, 1.10)	.12	31/80	1.12 (0.77, 1.64)	.30
Alpha-N-Phenylacetyl-L-glutamine	73/97	1.37 (0.97, 1.95)	.07	.41	30/97	2.77 (1.64, 5.01)	<.001	**.005**	22/97	2.48 (1.43, 4.63)	.002	**.02**		72/80	0.87 (0.56, 2.60)	.23	48/80	0.91 (0.65, 1.27)	.32	31/80	0.79 (0.54, 1.16)	.16
Alpha-Linolenic acid	73/97	1.09 (0.78, 1.53)	.62	.84	30/97	0.37 (0.21, 0.62)	<.001	**.006**	22/97	0.30 (0.14, 0.55)	<.001	**.01**		72/80	0.81 (0.80, 1.84)	.13	48/80	0.53 (0.36, 0.78)	**.003**	31/80	0.62 (0.41, 0.92)	**.02**
sn-2 LysoPC(18:1)	73/97	1.30 (0.95, 1.81)	.10	.41	30/97	0.26 (0.12, 0.52)	<.001	**.006**	22/97	0.24 (0.10, 0.54)	.001	**.02**		72/80	0.66 (1.16, 1.23)	.01	48/80	0.76 (0.55, 1.07)	.09	31/80	0.90 (0.62, 1.31)	.32

Metabolite	No.	OR (95% CI)	P	FDR	No.	OR (95% CI)	P	P	No.	OR (95% CI)	P	P		No.	OR (95% CI)	P	No.	OR (95% CI)	P	No.	OR (95% CI)	P
Indoxyl sulfate	73/97	1.28 (0.93, 1.79)	.13	.41	30/97	2.42 (1.47, 4.31)	.001	**.01**	22/97	2.66 (1.51, 5.19)	.002	**.02**		72/80	0.95 (0.35, 4.08)	.37	48/80	0.87 (0.63, 1.20)	.23	31/80	0.88 (0.61, 1.28)	.29
Phenylalanyl-Serine	73/97	1.55 (1.04, 2.45)	.04	.37	30/97	0.44 (0.25, 0.70)	.001	**.01**	22/97	0.44 (0.24, 0.74)	.004	**.03**		72/80	1.21 (0.71, 2.10)	.17	48/80	2.02 (1.39, 2.93)	**.001**	31/80	1.76 (1.18, 2.64)	**.01**
Linoleic acid	73/97	1.12 (0.80, 1.56)	.51	.76	30/97	0.43 (0.24, 0.72)	.002	**.02**	22/97	0.32 (0.15, 0.60)	.001	**.02**		72/80	0.80 (0.82, 1.74)	.11	48/80	0.56 (0.38, 0.81)	**.01**	31/80	0.63 (0.42, 0.96)	**.04**
L-Alanine	73/97	0.65 (0.42, 0.98)	.04	.37	30/97	1.87 (1.26, 2.88)	.003	**.02**	22/97	1.75 (1.15, 2.78)	.01	.07		72/80	1.17 (0.63, 2.28)	.19	48/80	0.93 (0.65, 1.34)	.38	31/80	1.31 (0.86, 2.00)	.14
N-Acetylglutamine	73/97	0.83 (0.52, 1.26)	.39	.66	30/97	1.71 (1.19, 2.52)	.005	**.03**	22/97	1.90 (1.27, 2.94)	.003	**.02**		72/80	0.84 (0.68, 2.12)	.17	48/80	0.92 (0.66, 1.27)	.33	31/80	0.92 (0.64, 1.33)	.35
Palmitic acid	73/97	1.23 (0.89, 1.71)	.22	.51	30/97	0.47 (0.26, 0.79)	.007	**.04**	22/97	0.32 (0.15, 0.62)	.002	**.02**		72/80	0.70 (1.11, 1.30)	.02	48/80	0.56 (0.38, 0.82)	**.01**	31/80	0.67 (0.44, 1.03)	**.04**
sn-2 LysoPC(20:2)	73/97	1.34 (0.98, 1.85)	.07	.41	30/97	0.39 (0.19, 0.74)	.007	**.04**	22/97	0.37 (0.16, 0.76)	.01	.07		72/80	0.72 (1.09, 1.28)	.03	48/80	0.72 (0.50, 1.04)	.07	31/80	0.80 (0.53, 1.21)	.19
L-Glutamine	73/97	0.80 (0.58, 1.11)	.19	.47	30/97	0.52 (0.31, 0.81)	.008	**.04**	22/97	0.60 (0.35, 0.98)	.05	.19		72/80	0.80 (0.82, 1.81)	.12	48/80	0.79 (0.58, 1.08)	.11	31/80	0.69 (0.49, 0.96)	**.03**
Isocitric acid	73/97	1.20 (0.85, 1.71)	.30	.59	30/97	1.96 (1.23, 3.33)	.008	**.04**	22/97	1.98 (1.18, 3.60)	.01	.07		72/80	1.21 (0.64, 2.47)	.20	48/80	0.92 (0.68, 1.24)	.32	31/80	0.89 (0.63, 1.24)	.28
Arachidonic acid	73/97	1.35 (0.98, 1.88)	.07	.41	30/97	0.51 (0.28, 0.85)	.01	**.05**	22/97	0.42 (0.20, 0.78)	.01	.07		72/80	0.88 (0.58, 2.44)	.22	48/80	0.70 (0.50, 0.99)	**.05**	31/80	0.83 (0.57, 1.21)	.21

[a] Individual metabolites associated with GC in the discovery stage (FDR<0.05) and validation stage (*P*<0.05) are shown here. Logistic regression analyses were conducted adjusting for age, sex, and *H.pylori* infection. Metabolites were ranked by the *P* values for GC in the discovery set. Four metabolites significantly associated with GC in the discovery set (FDR<0.05), including L-palmitoylcarnitine, stearoylcarnitine, sn-2 LysoPC(20:5) and sn-2 LysoPC(22:5), were found below the detection limit in the validation set, leaving the remaining 18 metabolites for analysis.

[b] We defined subjects with HGIN as early GC cases here.

CAG, chronic atrophic gastritis; GC, gastric cancer; IM, intestinal metaplasia; LGIN, low-grade intraepithelial neoplasia; OR, odds ratio; SG, superficial gastritis

eTable 3. Metabolite set enrichment analysis for plasma metabolites associated with gastric cancer

Metabolite Set	P	Fold enrichment	Metabolite
Glutamate Metabolism	**.002**	3.57	Gamma-Aminobutyric acid, L-Glutamic acid, L-Alanine, L-Aspartic acid, Pyruvic acid, Succinic acid, L-Glutamine
Alpha Linolenic Acid and Linoleic Acid Metabolism	**.006**	5.00	Linoleic acid, Arachidonic acid, Alpha-Linolenic acid
Arachidonic Acid Metabolism	**.04**	5.00	L-Glutamic acid, Arachidonic acid
Phenylacetate Metabolism	**.04**	5.00	L-Glutamine, Alpha-N-Phenylacetyl-L-glutamine
Malate-Aspartate Shuttle	**.04**	5.00	L-Glutamic acid, L-Aspartic acid
Urea Cycle	**.04**	2.50	L-Glutamic acid, L-Alanine, L-Aspartic acid, Pyruvic acid, Urea, L-Arginine, L-Glutamine, Citrulline
Beta-Alanine Metabolism	**.05**	3.00	Dihydrouracil, L-Glutamic acid, L-Aspartic acid, Pantothenic acid, Uracil
Ammonia Recycling	**.05**	3.00	L-Glutamic acid, L-Asparagine, L-Aspartic acid, Pyruvic acid, L-Glutamine
Purine Metabolism	.09	2.50	L-Glutamic acid, Hypoxanthine, L-Aspartic acid, Uric acid, Xanthine, L-Glutamine
Aspartate Metabolism	.09	2.50	L-Glutamic acid, L-Asparagine, L-Aspartic acid, L-Arginine, L-Glutamine, Citrulline
Tyrosine Metabolism	.10	3.33	L-Glutamic acid, L-Tyrosine, L-Aspartic acid
Glutathione Metabolism	.10	3.33	L-Glutamic acid, L-Alanine, Pyroglutamic acid
Amino Sugar Metabolism	.10	3.33	L-Glutamic acid, Pyruvic acid, L-Glutamine
Pyrimidine Metabolism	.10	3.33	Dihydrouracil, Uracil, L-Glutamine
Nicotinate and Nicotinamide Metabolism	.10	3.33	L-Glutamic acid, L-Glutamine, N1-Methyl-2-pyridone-5-carboxamide
Alanine Metabolism	.10	3.33	L-Glutamic acid, L-Alanine, Pyruvic acid

Glucose-Alanine Cycle	.18	2.50	D-Glucose, L-Glutamic acid, L-Alanine, Pyruvic acid
Steroid Biosynthesis	.20	5.00	Palmitic acid
Selenoamino Acid Metabolism	.20	5.00	L-Alanine
Glycerolipid Metabolism	.20	5.00	Palmitic acid
Histidine Metabolism	.20	5.00	L-Glutamic acid
Folate Metabolism	.20	5.00	L-Glutamic acid
Fatty Acid Elongation In Mitochondria	.20	5.00	Palmitic acid
Fatty Acid Biosynthesis	.20	5.00	Palmitic acid
Warburg Effect	.25	1.67	Citric acid, D-Glucose, L-Glutamic acid, L-Malic acid, L-Lactic acid, Isocitric acid, Pyruvic acid, Succinic acid, L-Glutamine
Tryptophan Metabolism	.26	2.00	L-Glutamic acid, L-Alanine, 5-Hydroxy-L-tryptophan, L-Kynurenine, L-Tryptophan
Cysteine Metabolism	.36	2.50	L-Glutamic acid, Pyruvic acid
Propanoate Metabolism	.36	2.50	L-Glutamic acid, L-Valine
Lysine Degradation	.36	2.50	L-Glutamic acid, L-Lysine
Phenylalanine and Tyrosine Metabolism	.50	1.67	L-Glutamic acid, L-Tyrosine, L-Phenylalanine
Bile Acid Biosynthesis	.50	1.67	Glycocholic acid, Palmitic acid, Taurine
Fatty acid Metabolism	.50	1.67	L-Carnitine, Palmitic acid, L-Palmitoylcarnitine
Glycine and Serine Metabolism	.51	1.25	Betaine, Creatine, L-Glutamic acid, L-Alanine, L-Threonine, Pyruvic acid, L-Arginine, L-Methionine
Arginine and Proline Metabolism	.51	1.25	Creatine, L-Glutamic acid, L-Proline, L-Aspartic acid, Succinic acid, Urea, L-Arginine, Citrulline
Valine, Leucine and Isoleucine Degradation	.76	0.83	Alpha-ketoisovaleric acid, L-Glutamic acid, L-Isoleucine, Succinic acid, L-Leucine, L-Valine

Citric Acid Cycle	.76	0.83	cis-Aconitic acid, Citric acid, L-Malic acid, Isocitric acid, Pyruvic acid, Succinic acid

P values of over-representation analysis were calculated using hypergeometric test; Fold enrichment was calculated by dividing the observed number of

significant metabolites by the expected number of significant metabolites for each pathway.