Circulating Metabolomic Profiling Detects Novel Markers of Myocardial Infarction among Cardiac-chest Pain Cases

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Abstract

Background: A unique human plasma metabolic pattern of myocardial infarction (MI) has been recognized for over a decade. The discriminant metabolites are potential diagnosis and warning markers, and have connections with traditional cardiovascular risk factors and circulating transcriptomics.

Methods: A total of 146 chest pain (85 MIs and 61 non-MI chest pain cases) and 84 control individuals were recruited. GC/MS and LC/MS were applied to identify and quantify the plasma metabolites. The metabolites were analyzed by multivariate statistical analysis to describe different patterns of CADs with chest pain. Receiver operating characteristic (ROC) and odds ratio (OR) were calculated to identify potential markers of MI cases, and the markers were further evaluated based on human circulating transcriptomics from the GEO database. The correlation of metabolites to clinical indices was assessed.

Results: MI can be clearly distinguished from non-MI chest pain cases by the metabolites we identified, better than by medical history combined with routine biochemical parameters. Pathway analysis highlighted an upregulated methionine metabolism and downregulated arginine biosynthesis that were deeply involved in MI cases. Among all the metabolites, deoxyuridine (dU) and methionine scored highly in ROC (>0.91) and are related to LDH and AST ($p<0.05$). OR values ($\log OR>2$) suggested, with or without adjusting by lipid levels, age, smoking, diabetes and hypertension, that dU and methionine are risk factors for alerting about MI occurrence. Transcriptomics data showed elevations of plasma CDA and UPP1, and a decrease of cardiac MARS, which are consistent with the increased levels of plasma dU and methionine, respectively.

Conclusions: For the first time, dU and methionine were suggested as markers for MI diagnosis and as high-risk markers to alert about an onset of MI. Plasma metabolite changes are not only directly related to cardiac damage but also could be related to circulating cells.

1. Background

A strangling feeling in the chest is a typical manifestation of coronary artery disease (CAD). In most cases, CAD develops as plaque builds up on the artery walls. When it progresses to myocardial infarction (MI), coronary heart disease will be life-threatening and extremely dangerous in the ensuing days or weeks due to its various fatal complications. However, chest pain can be caused by other cardiovascular events (e.g., unstable angina) or other, non-cardiac cases (e.g., gastroesophageal reflux disease). Thus, noticing early warning signs to alert patients about the onset of MI, taking precautions and recognizing MI are among the primary focuses of the management of atherosclerotic coronary artery disease, which is the main cause of coronary heart diseases.

Diagnostic and predictive biomarkers of MI are of crucial importance. Among all cardiac damage biomarkers, such as CK-MB, AST and cardiac troponins (cTnt), cTnt is the preferred MI biomarker clinically. However, some studies showed that an unexpected elevation of this cardiomyocyte protein was observed without obvious connection to cardiac injury. Hence, clinicians need to carefully determine whether troponin elevation is direct proof of myocardial necrosis, especially when the underlying reason for its release is unclear. [1–3]

More potential biomarkers are urgently needed to facilitate the diagnosis of, as well as for preferably alerting about and predicting MI onset. CAD is a lifestyle disease, and the abnormal abundance of circulating metabolites surely reflects the metabolic state of CAD patients. Medical history records and laboratory tests, e.g., sex, age, smoking history, function of the primary organs, and biochemical assays, are important factors worthy of consideration in identifying diagnostic markers and clarifying the underlying mechanism involved. Since ischemic heart diseases are characterized by profound metabolic shifts at both the circulatory and local levels[4], metabolomics has been applied to study the metabolic pattern changes detected in the plasma or serum of CAD patients. Early in 2002, a pioneering work was published showing that NMR-based metabonomics had the potential to rapidly and noninvasively diagnose the presence and severity of coronary heart disease[5]. In 2005, Marc Sabatine and his colleagues identified metabolic biomarkers of myocardial ischemia associated with physical exercise[6]. Later studies focused on identifying biomarkers and metabolic pathways and exploring the underlying mechanisms associated with cardiovascular diseases[7–13]. Excitingly, a panel of potential markers has been suggested for coronary heart diseases, such as arginine and homocysteine, and the underlying mechanisms of their action have been explored[14–16]. Unfortunately, few publications have focused on the diagnosis and prediction of MI onset among CAD patients with chest pains, although the occurrence of MI is deeply influenced by whether an individual has traditional risk factors, such as male sex, diabetes, tobacco smoking and hypertension.

In this study, a metabolomic platform with GC/MS and LC/MS instrumentation was employed to profile plasma metabolites of hospitalized CAD patients with chest pain (including MI and non-MI chest pain cases) and their controls. Metabolic patterns were evaluated, and metabolic markers were screened and described based on semi-quantitative data, AUC, odds ratios (OR)[17] and their connections with well-recognized cardiovascular disease risk factors. We aimed to identify metabolic markers to facilitate the diagnosis and early prediction of MI among chest pain patients. The mechanisms behind the biomarker changes are also briefly discussed.

2. Methods

2.1 Human plasma samples

A total of 146 chest pain cases highly suspected of MI, and 84 individual controls were recruited from October, 2017 to March, 2018, with exclusions criteria of fever, hepatic decompensation, renal failure, cancers, endocrine and hematological diseases. The blood samples of chest pain cases were

collected within 48 hours since the symptom was first reported and fasting for at least 6 hours. All control cases were fasting for at least 6 hours too. After routine diagnostic procedures, including evidence from ECGs, serum infarction biomarkers, computed tomography angiography or coronary angiography, 85 were later confirmed to be NSTEMI or STEM as the MI cases (MIs) and the remaining 61 were confirmed to be non-MI chest pain cases (non-MIs). All the non-MIs include 34 unstable angina (UA) cases, and 27 other non-MI cardiac cases (non-MICs), including myocarditis, valvular heart diseases etc.

The venous blood samples were collected from fasting state volunteers in EDTA-Na anticoagulated tubes in the morning. Within 2 hours, blood samples were centrifuged at 1000 *g* for 5 min, and each of the supernatant plasma was transferred to another tube, frozen at -80°C in refrigerator. Before use of the plasma samples, they were thawed by incubation at 37 °C bath for 15 min, vortexed and centrifuged at 650 *g* for 5 min.

2.2 Equipment for blood examinations

The instrument blood count and blood biochemistry, model xn-10, is made in Hyogo, Japan. Both NT-proBNP and serum infarction marker (cTns) are Roche company of Germany, model Cobas 6000, produced in Mannheim, Germany. The biochemical instrument is Beckman Coulter of the United States, model au5800, produced in Shizuoka, Japan.

2.3 Chemicals and reagents

Stable isotope internal standard 5-¹³C-glutamine was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Myristic-1,2-¹³C₂ acid, methoxamine hydrochloride (purity 98%) and pyridine (≥ 99.8% GC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-methyl-trimethylsilyl-trifluoroacetamide (MSTFA) plus 1% trimethylchlorosilane (TMCS) were provided by Pierce Chemical (Rockford, IL, USA). Methanol, acetonitrile and n-heptane were HPLC grade and obtained from Merck (Darmstadt, Germany). Purified water was produced by a Milli-Q system (Millipore, Bedford, MA, USA). Ammonium acetate (purity 98.0%) and ammonia solution (25%, w/w) were purchased from Aladdin (Shanghai, China) and Nanjing Chemical Reagent (Nanjing, China), respectively.

2.4 GC/MS and UPLC-QTOF/MS analysis

A well-developed metabolomic platform was utilized to profile metabolites in plasma samples of the clinic patients. For the analysis in GC/MS system((Shimadzu GCMS-QP2010 Ultra, Kyoto, Japan), the plasma samples were pretreated, extracted, and derivatised in a similar way to that described previously[18], Method S1. To minimize systematic variations, the samples were randomly analyzed with the quality control (QC) samples inserted, and the intensity of internal standard(IS), myristic-1,2-¹³C₂ acid, and an external reference standard, methyl myristate was evaluated. The raw GC/MS data were processed using a post-run analysis software(Shimadzu GC/MS solution 2.0), each of the metabolite was identified and the quantitative data was acquired in the same way as reported[19].

For the analysis in UPLC-QTOF/MS system (AB SCIEX TripleTOF® 5600, Foster City, CA), the plasma samples were pretreated in the same way as in GC/MS analysis with a few modifications, which used the other internal standard of 5-¹³C-glutamine dissolved in methanol at 15 µg/ml. The UPLC-QTOF/MS analysis was carried out as previously reported[20]. The detail information and protocol for GC/MS and UPLC-QTOF/MS is available in the Supplementary material, method S2.

2.5 Multivariate statistical analysis

After acquirement of the data in GC/MS and UPLC-QTOF/MS, the detected peaks/identified metabolites in each sample were aligned, quantitated, and all the samples data constructed a data matrix, where the missing data were excluded as default during analysis. After normalization against the IS, the data was evaluated using SIMCA P14.1 software (Umetrics, Umeå, Sweden) [19], Method S3. Concisely, principal component analysis(PCA), partial least square to latent structure discriminant analysis (PLS-DA), orthogonal PLS-DA(OPLS-DA) models were calculated to show the clustering or separation of samples from different groups For PLS-DA modeling, samples from the different groups were classified such that all samples were divided into different groups (e.g., MIs, non-MIs, controls, etc.) as the qualitative 'dummy' variables, Y. The goodness of fit for the models was evaluated using three quantitative parameters: R²X and R²Y were the explained variation in X and Y, respectively, and Q²Y was the predicted variation in Y. Permutation test was assessed for model validation, where the higher level of R²Y and Q²Y, and lower value of the intercept of R² (lower than 0.2) and Q² (lower than 0.0) suggested good model and prediction ability.

2.6 Discriminant metabolites and statistical analysis

After normalization against the IS, all the semi-quantitative data from both GC/MS and UPLC-QTOF/MS was logarized so that the state probabilities of the data queue tended to a normal distribution. The discriminant metabolites between groups were screened and chosen based on Variable Importance (VIP) using SIMCA-P 14.1, and the independent sample t-test of the logarized data using SPSS (version 23.0, SPSS Inc., Chicago, IL, US).

Metabolic pathway enrichment and topology analyses were performed using Metaboanalyst 3(<https://www.metaboanalyst.ca/>), Method S4. The KEGG ID of discriminatory compounds were uploaded and embedded in human pathway library for pathway analysis and hypergeometric tests, with the pathway analysis algorithms of Fisher's Exact Test, a pathway topology algorithms of Relative-betweenness Centrality, and KEGG pathway library version of Homo sapiens. Potential biomarkers were identified based on the identified metabolic pathways in databases such as *KEGG* (<http://www.genome.jp/kegg/>), human metabolomic data base (HMDB), (<http://www.hmdb.ca/>) and *LipidMaps* (<http://www.lipidmaps.org/>).

For the data inconformity with normal distribution from clinical assaying, a nonparametric test (Mann-Whitney U test, two-sided) was employed to evaluate statistical significance. ROC analysis and OR calculation were performed using SPSS as well. Before computing OR value, the logarized data of a metabolite(i) for each subject of ORi was normalized by subtracting mean value of ORmean within this group, and then divided by the standard deviation(SD) within the group, shown as the normalized ORs=(ORi-ORmean)/SD.

2.7 Transcriptomics data

We studied open access transcriptomics data from GEO. The human myocardial infarction plasma data are from GEO accession GSE48060. Mice myocardial infarction data are from GSM12346. Data were directly plotted without transformation.

3. Results

3.1 Clinical description of chest pain individuals by PCA and OPLS-DA plots

Tables 1 and 2 show the history examinations and basic laboratory tests of the volunteers. Generally, higher glucose, AST, LDH, HBDH, and CK levels and lower ALB and Ca^{2+} concentrations were detected in the chest pain patients than the control group. Among the 146 chest pain inpatients, approximately 65% had taken aspirin and statin treatments before blood collection. As a result, TC, TG, LDL-c and HDL-c levels were all lower in chest pain inpatients than the controls.

Table 1
Sample Characteristics: Controls vs all the chest pain cases

Clinical concerns	Variables	The controls	The chest pain cases	Statistics
		n = 84	n = 146	p Values
Demographics	Male	56	99	0.26
	Age(years)	50.25 ± 1.76	59.28 ± 1.83	0.00
Cardiac risk factors	Hypertension	32	83	0.00
	Diabetes	8	36	0.00
	TC(mmol/L)	4.93 ± 0.15	4.08 ± 0.11	0.00
	TG(mmol/L)	1.43 ± 0.12	1.51 ± 0.08	0.17
	LDL-C(mmol/L)	3.15 ± 0.11	2.70 ± 0.08	0.00
	HDL-C(mmol/L)	1.31 ± 0.04	0.95 ± 0.02	0.00
	LPa(mg/L)	349.35 ± 51.45	339.39 ± 27.47	0.65
	Tobacco using	8	43	0.00
	Drinking history	0	16	0.00
Cardiovascular medications	Aspirin	1	88	0.00
	Statin therapy	6	94	0.00
	β-blockers	0	7	0.00
Prior cardiovascular disease		2	22	0.01
Biochemical items	ALT(U/L)	27.15 ± 2.48	39.33 ± 2.90	0.00
	AST(U/L)	26.31 ± 1.45	77.35 ± 9.78	0.00
	ALP(U/L)	78.20 ± 2.19	86.16 ± 2.26	0.03
	GGT(U/L)	30.69 ± 2.75	45.18 ± 3.80	0.02
	LDH(U/L)	169.30 ± 3.85	398.72 ± 32.22	0.00
	CK(U/L)	119.30 ± 11.27	480.97 ± 78.58	0.94
	HBDH(U/L)	107.80 ± 3.87	297.38 ± 28.85	0.00
	TBIL(μmol/L)	14.36 ± 0.56	13.74 ± 0.71	0.03
	DBIL(μmol/L)	4.51 ± 0.28	4.96 ± 0.26	0.83
	IBIL(μmol/L)	9.16 ± 0.42	8.77 ± 0.48	0.05
	TP(g/L)	70.80 ± 0.47	61.12 ± 0.48	0.00
	ALB(g/L)	44.68 ± 0.54	35.80 ± 0.36	0.00
	GLB(g/L)	25.78 ± 0.48	25.33 ± 0.39	0.14
	ALB/GLB	1.79 ± 0.05	1.45 ± 0.03	0.00
	GLU(mmol/L)	5.77 ± 0.13	6.01 ± 0.22	0.23
	Urea(mmol/L)	5.13 ± 0.18	7.37 ± 0.52	0.00
	Cr(μmol/L)	71.14 ± 2.00	102.13 ± 11.16	0.00
	UA(μmol/L)	325.57 ± 9.61	378.91 ± 13.24	0.04
	Ca(mmol/L)	2.36 ± 0.02	2.17 ± 0.01	0.00
Values are presented as Mean ± SE. Mann-Whitney U test was applied to produce P value.				

Table 2
Sample Characteristics: MI vs non-MI chest pain cases

Clinical concerns	Variables	Controls	Chest pain cases		Statistics
		(n = 84)	non-MI(n = 61)	MI(n = 85)	p values
Demographics	Male	56	32	67	0.00
	Age(years)	50.25 ± 1.76	60.18 ± 2.42	65.08 ± 1.60	0.12
Cardiac risk factors	Hypertension	32	32	51	0.28
	Diabetes	8	8	28	0.01
	Tobacco using	8	8	35	0.00
	TC(mmol/L)	4.93 ± 0.15	3.69 ± 0.12	4.28 ± 0.15	0.01
	TG(mmol/L)	1.43 ± 0.12	1.26 ± 0.09	1.65 ± 0.11	0.02
	LDL-C(mmol/L)	3.15 ± 0.11	2.40 ± 0.09	2.86 ± 0.11	0.01
	HDL-C(mmol/L)	1.31 ± 0.04	0.96 ± 0.03	0.94 ± 0.03	0.70
	LPa(mg/L)	349.35 ± 51.45	334.55 ± 47.64	341.96 ± 33.82	0.56
Prior cardiovascular disease		2	12	10	0.24
Serum biomarkers	cTNT(ng/ml)	-	756.13 ± 242.59	1559.30 ± 276.64	0.56
	CK-MB(U/L)	-	24.98 ± 6.08	32.10 ± 5.89	0.68
	Mb(ng/ml)	-	59.30 ± 23.79	66.99 ± 15.32	0.46
Biochemical items	ALT(U/L)	27.15 ± 2.48	32.17 ± 4.59	43.14 ± 3.65	0.04
	AST(U/L)	26.31 ± 1.45	29.12 ± 2.91	102.99 ± 14.10	0.00
	LDH(U/L)	169.30 ± 3.85	213.05 ± 10.22	497.43 ± 45.36	0.00
	CK(U/L)	119.30 ± 11.27	86.83 ± 12.99	690.51 ± 113.49	0.00
	HBDH(U/L)	107.80 ± 3.87	132.79 ± 6.71	384.89 ± 40.81	0.00
	TP(g/L)	70.80 ± 0.47	61.95 ± 0.71	60.68 ± 0.62	0.18
	GLU(mmol/L)	5.77 ± 0.13	5.49 ± 0.22	6.28 ± 0.32	0.06
	Ca(mmol/L)	2.36 ± 0.02	2.20 ± 0.02	2.16 ± 0.02	0.11
Values are presented with Mean ± SE. Mann-Whitney U test was applied to produce P value between MI and non-MI chest pain cases.					

Based on clinical parameters (listed in Table 1 “variables”), including “biochemical items”, “demographics” and “cardiac risk factors”, an unsupervised PCA score plot was created. The model indicated a few outliers when the samples were either divided into 3 (controls, MIs, non-MIs) or 4 groups (controls, UA, MIs and other non-MICs), and each of the groups generally overlapped with the others (Fig. 1A1, 1A2). However, a supervised PLS-DA revealed a visible separation of the groups with only a little overlap when the samples were divided into 3 groups, i.e., MIs, non-MIs and controls. When the samples were divided into 4 groups (MIs, UAs, other non-MICs, and controls), the controls, UAs and MIs were fairly well separated, but the other non-MICs primarily showed overlaps with UAs and MIs (Fig. 1A3, 1A4). These findings suggest that the model was not powerful at differentiating non-MICs from MIs and UAs based on basic laboratory tests and history examinations.

3.2 Plasma metabolomic description of chest pain individuals by PCA and OPLS-DA plots

GC/MS and LC/MS analysis of the plasma samples aligned the metabolites in typical chromatograms (Figure S1-2). Deconvolution of the GC/MS chromatograms produced 135 independent peaks from the plasma samples, 83 of which were authentically identified as metabolites; LC/MS produced 279 peaks, and 76 metabolites were identified (Table S1-2). Quantitative data were acquired for each metabolite in the plasma samples of the control, MI, UA and other non-MI cardiac cases.

Based on the metabolomic data derived from GC/MS and LC/MS analysis, the PCA score plot again showed a few outliers when the samples were divided into 3 or 4 groups, as indicated above. Unlike with the clinical data, unsupervised PCA analysis of metabolomic data showed that the majority of MIs deviated from the others, regardless of whether the 3 or 4 groups were defined, although the control, non-MICs and UAs overlapped with each other to some extent (Fig. 1B1, 1B2). The supervised PLS-DA model revealed that samples from each group clustered closely and anchored away from the other groups when the samples were divided into 3 groups (Fig. 1B3). When the samples were divided into 4 groups, the majority of MIs and controls clustered separately, while the UAs and non-MICs primarily overlapped with each other, with a minority overlapping with MIs and controls (Fig. 1B4). The distant

separation of MIs from the other groups suggested distinctly different metabolic patterns between MIs and the groups of UA and non-MICs, while the overlapping of the groups suggested similar plasma metabolic patterns between UA and the other non-MICs. In general, metabolomic data better characterized MIs than history examinations and laboratory tests, and the score plot of non-MI chest pain cases (including UA and non-MI cardiac cases) indicated that they had moderate metabolic perturbation relative to the MI cases because they anchored between MI and the controls (Fig. 1B3). The above data suggest that subgroups of MI can be recognized by multivariate analysis of identified plasma metabolites more effectively than by routine clinical parameters.

3.3 Pathway analysis of differential metabolites

OPLS-DA analysis showed different metabolomic patterns of the non-MIs from the controls (Fig. 2A1). Statistical analysis suggested 50 discriminant metabolites ($p < 0.05$) that differentiated non-MI chest pain inpatients from the controls (Table 3). Similarly, MI cases primarily showed different metabolomic patterns from non-MIs (Fig. 2B1). According to the statistical analysis and the VIP values, 54 discriminant metabolites were identified between MIs and non-MIs (Table 3).

Table 3
Discriminant metabolites list: non-MIs vs controls and MIs vs non-MIs

Differential metabolites	Controls (n = 84)		MI cases (n = 85)		non-MI cases (n = 61)		MI vs non-MI		non-MI vs Con	
	Mean	SE	Mean	SE	Mean	SE	FC	T-test	FC	T-test
Deoxyuridine	26212	9533	1025278	150576	16840	555	60.885	***	0.642	/
Adenosine phosphosulfate	51723	4141	99104	8542	42213	3793	2.348	***	0.816	/
Deoxyadenosine monophosphate	310296	7783	102462	13401	311056	7624	0.329	***	1.002	/
Guanosine diphosphate	7379	469	27614	4153	6648	530	4.154	***	0.901	/
Inosine 2'-phosphate	14875	683	37785	4687	14988	809	2.521	***	1.008	/
Adenosine monophosphate	20124	1044	25648	1689	19902	1016	1.289	***	0.989	/
Hypoxanthine	705034	24192	373454	29693	734901	52065	0.508	***	1.042	/
Glycolate	8319	247	9121	331	7620	306	1.197	**	0.916	/
Methionine	113239	2830	335994	27792	109192	2631	3.077	***	0.964	/
Arginine	810028	22688	169566	34842	731551	31282	0.232	***	0.903	/
Valine	2334562	53682	3710196	209295	2425637	63465	1.530	***	1.039	/
Citrulline	422244	11079	296302	13267	469763	20870	0.631	***	1.113	/
Shikimate	14871	796	8837	491	15072	782	0.586	***	1.014	/
Ornithine	747344	19685	522218	23919	805398	34957	0.648	***	1.078	/
Alanine	402892	10006	663687	37026	446350	16079	1.487	***	1.108	/
Glycine	145111	2764	256201	15108	150041	3152	1.708	***	1.034	/
Homocysteine	7517	382	20169	2293	7693	504	2.622	***	1.023	/
Aspartate	155030	10889	194713	12674	123523	10287	1.576	***	0.797	/
Ribose	223498	12602	340299	15678	254617	23072	1.337	**	1.139	/
1-Monopalmitin	30533	1705	33208	1616	25699	1904	1.292	**	0.842	/
Glycerate	125449	4077	54424	3823	127894	10153	0.426	***	1.019	/
Citrate	6687162	203004	1395841	280056	6186805	304233	0.226	***	0.925	/
NAD+	11766	301	25536	3262	11479	330	2.225	***	0.976	/
NADPH	5344	269	35996	7862	4157	192	8.659	***	0.778	###
Uracil	146594	7319	60619	9826	319956	18480	0.189	***	2.183	###
Xanthine	161018	5234	99621	11423	234923	11566	0.424	***	1.459	###
Adenosine	33452	2133	31326	3112	18676	1948	1.677	***	0.558	###
IDP	3112579	73422	1512965	153666	3597655	92953	0.421	***	1.156	###
Adenine	15026	2519	13092	2292	5543	393	2.362	**	0.369	###
Succinate	88550	1816	36686	3233	79439	2388	0.462	***	0.897	##
Malate	61898	2263	35918	4299	78778	4163	0.456	***	1.273	###
2-Ketoglutarate	33628	1314	21806	2264	78634	4864	0.277	***	2.338	###
Acetoacetate	132245	2671	235115	13087	160911	7415	1.461	***	1.217	###
Carbamoylphosphate	29690	1819	23063	2179	36316	1532	0.635	***	1.223	##
Dihydroorotate	33819	1003	31876	2619	22310	579	1.429	***	0.660	###
Pantothenate	54720	3022	38266	4405	83814	4827	0.457	***	1.532	###

The data was not logarized, and expressed as Mean $\pm$ SE. Fold change (FC) were calculated by the original, non-logarized data directly. Statistical significance was evaluated by using T-test with equal-variance of two tails, after ANOVA assessment of the variance, ***, **, *: $p < 0.001, 0.01, 0.05$, respectively, between MI and non-MI chest pain cases; ###, ##, #: $p < 0.001, 0.01, 0.05$, respectively, between non-MI chest pain cases and the controls. '/' represents the statistical significance of p values more than 0.05.

Differential metabolites	Controls (n = 84)		MI cases (n = 85)		non-MI cases (n = 61)		MI vs non-MI		non-MI vs Con	
Phenylpyruvate	51177	955	63363	2279	56537	1514	1.121	*	1.105	##
Cysteine	106041	3333	68660	3639	150632	7856	0.456	***	1.421	###
Isoleucine	1694252	47827	4794207	400231	2264556	105757	2.117	***	1.337	###
Serine	187997	4449	345414	14192	211966	5374	1.630	***	1.127	###
Proline	805696	27760	1281948	56175	912165	3390	1.405	***	1.132	#
Threonine	661526	23707	1415808	70560	574241	19440	2.466	***	0.868	##
Phenylalanine	1051097	31497	1757945	133629	2349797	58409	0.748	***	2.236	###
Glutamine	6299813	30803	6265669	37518	6115270	38706	1.025	**	0.971	###
Histidine	3756407	54216	2876183	73183	3370631	66123	0.853	***	0.897	###
Taurine	851621	35791	1382464	89177	727822	36862	1.899	***	0.855	#
Lysine	1733874	38073	505537	102029	2002743	55294	0.252	***	1.155	###
N-Acetylmethionine	329453	18159	68946	14885	265119	11352	0.260	***	0.805	##
Cytosine	7058	102	5624	153	6609	135	0.851	***	0.936	##
3-Phospho-Serine	11045	654	17870	1469	8893	284	2.009	***	0.805	##
Homoserine	661526	23707	1415808	70560	574241	19440	2.466	***	0.868	##
1-Monostearin	17548	911	19128	896	13626	841	1.404	***	0.777	##
2-Dehydro-D-Gluconate	47777	2930	21494	3999	90849	4764	0.237	***	1.902	###
Oxalate	44511	2459	48639	3025	27020	2378	1.800	***	0.607	###
Tryptophan	647430	23608	1366119	100129	1584205	58633	0.862	/	2.447	###
Glutamate	253094	9911	384958	35729	384612	16588	1.001	/	1.520	###
Hydroxyproline	26782	1974	17148	1951	17583	2030	0.975	/	0.657	##
Salicylic Acid	4870	1788	72167	12248	57320	7503	1.259	/	11.771	###
Pyruvate	121954	5245	183553	22838	184240	7253	0.996	/	1.511	###
Homocysteine	11932	344	14822	1109	13267	310	1.117	/	1.112	##
Cystathionine	10857	625	13618	1322	17869	1568	0.762	/	1.646	###
2-Hydroxybutyrate	279219	12481	421962	23064	405608	28986	1.040	/	1.453	###
3-Hydroxybutyrate	52649	3298	79377	10282	85759	13390	0.926	/	1.629	#
Gluconic Acid	105169	4261	289949	39667	203681	34215	1.424	/	1.937	##
Indole-3-Propanate	55659	16898	8531	1748	5464	801	1.561	/	0.098	##
Glycerol	400975	13803	290401	11659	303966	15733	0.955	/	0.758	###
Cholesterol	822261	17789	690461	14851	684060	16272	1.009	/	0.832	###
Alpha-Tocopherol	116918	2949	93627	2218	94284	2954	0.993	/	0.806	###
Deoxyadenosine	23123	1541	17568	2255	13041	1288	1.347	/	0.564	###
Thymine	544496	17903	518803	20345	457002	25215	1.135	/	0.839	##
Inosine	65657	2868	28268	3939	35665	2829	0.793	/	0.543	###
NADP+	24365	1068	23264	1803	19968	712	1.165	/	0.820	###
The data was not logarized, and expressed as Mean $\pm$ SE. Fold change (FC) were calculated by the original, non-logarized data directly. Statistical significance was evaluated by using T-test with equal-variance of two tails, after ANOVA assessment of the variance, ***, **, *: p < 0.001, 0.01, 0.05, respectively, between MI and non-MI chest pain cases; ###, ##, #: p < 0.001, 0.01, 0.05, respectively, between non-MI chest pain cases and the controls. '/' represents the statistical significance of p values more than 0.05.										

Of the 50 metabolites differentiating non-MIs from the controls, levels of gluconic acid and isoleucine were higher in non-MIs, while succinate, inosine, and arginine were lower, and all the above metabolites deviated further in MIs as the cardiac damage became more severe (Fig. 3C, 3D). These findings indicate that the above metabolites are involved in the development of cardiac damage.

Although glycerol, salicylic acid, and deoxyadenosine showed significant differences between the non-MI and control groups, they had no significant difference between the MI and non-MI cardiac groups. These are thus suggested as markers of non-MI chest pain. In addition to endogenous metabolites, we found that salicylic acid, an exogenous metabolite, also characterized the group of chest pain cases. Salicylic acid is the primary metabolite of aspirin, and a review of inpatient information and clinical data revealed that a large portion of chest pain patients had taken aspirin for the management of CAD.

Moreover, deviated levels of dU, methionine, homoserine, etc. were only observed in MI cases, but not between the controls and non-MIs, indicating their association with the development of MI (Fig. 3B).

A Venn diagram was created to show the discriminant metabolites between MI and non-MIs and those between non-MIs and controls. The overlapping region (B) in the Venn diagram (Fig. 3E) lists the metabolites screened out in both of the two comparison groups, suggesting that they were mostly likely risk factors or markers of the occurrence and development of MI, reflecting homeostatic disturbance induced by myocardia hypoxia. Figure 3f shows the pathway analysis of the metabolites in the Venn A + B region (control vs non-MI), and Fig. 3G shows the pathway analysis of discriminant metabolites in the Venn B + C region (MI vs non-MI). Generally, arginine biosynthesis and pyrimidine metabolism were the most significantly altered metabolic pathways in non-MI chest pain patients' plasma compared to healthy individuals'. Enrichment and pathway analysis for the metabolites of the Venn C area by MetaboAnalyst showed that arginine biosynthesis ($p < 0.01$, FDR $< 1\%$) was the most altered metabolic pathway (Fig. 3H). Alanine, aspartate and glutamate metabolism ($p < 0.01$, FDR $< 1\%$) deserve attention in MI as well.

3.4 Methionine, dU and homoserine are candidate biomarkers for MI occurrence

A combined biosignature of homoserine, IDP and 2-ketoglutarate discriminated MI from non-MI chest pain inpatients with high accuracy (Fig. 3L,AUC = 0.98, sensitivity: 94.1%, specificity: 100%).

The potential capacity of each discriminant metabolite to diagnose MI was assessed by ROC analysis (Table 4). Notably, although pathway analysis did not draw our attention to the methionine-related metabolic module, methionine and homoserine showed their potential in distinguishing MI from non-MI cardiac cases. Homoserine (AUC = 0.94; specificity = 100%, sensitivity = 81%) was more specific for MI diagnosis but less sensitive than methionine (AUC = 0.96; specificity = 94.6%; sensitivity = 89.4%) (Fig. 3J). dU also scored highly, with an AUC over 90% (Fig. 3F). Some other metabolites that showed good diagnostic potential were cysteine, 2-ketoglutarate, IDP, and uracil (Table 4).

Table 4
Differential metabolites and the diagnostic potential between MI and non-MI chest pain cases

Differential metabolites	AUROC	95%C.I.	Sensitivity	Specificity	log(OR)	95%C.I.
Homoserine&IDP&α-Ketoglutarate	0.9810	0.9614-1.0000	94.10%	100%	3.02	2.12–3.93
Methionine	0.9643	0.9309–0.9978	89.40%	94.60%	3.48	2.01–4.94
Homoserine	0.9431	0.893–0.9932	80.90%	100%	1.61	1.04–2.18
α-Ketoglutarate	0.9390	0.8876–0.9905	100%	0.00%	-0.11	-0.28 --0.03
Uracil	0.9166	0.8585–0.9747	100%	0.00%	-2.38	-3.45 --1.32
Deoxyuridine	0.9166	0.8462–0.987	80.90%	100%	3.01	1.71–4.30
2-Dehydro-D-gluconate	0.9040	0.8362–0.9717	2.10%	100%	-1.69	-2.53 - -0.84
Cysteine	0.8976	0.8302–0.9651	100%	0.00%	-0.41	-0.61 - -0.20
Deoxyadenosine monophosphate	0.8976	0.8335–0.9618	100%	0.00%	-5.57	-8.61 --2.53
IDP	0.8838	0.8124–0.9553	100%	0.00%	-5.15	-7.86 --2.44
Glyceric acid	0.8758	0.8022–0.9493	100%	0.00%	-1.53	-2.08 --0.98
Citrate	0.8568	0.7774–0.9362	100%	0.00%	-1.69	-2.50 - -0.88
Succinate	0.8562	0.7768–0.9357	100%	0.00%	-1.48	-2.14 - -0.83
Pantothenate	0.8332	0.7452–0.9213	100%	0.00%	-0.98	-1.36 - -0.59
Xanthine	0.8240	0.7312–0.9168	2.10%	100%	-1.06	-1.46 - -0.65
Arginine	0.8235	0.7319–0.9151	2.10%	100%	-0.82	-1.10 - -0.53
Logarization transformation of the data was applied to the data before the calculation.						

Moreover, to assess the role of these metabolites as risk factors for the prediction of MI occurrence, OR values were calculated between the MI and non-MI groups. Homoserine, dU and methionine had high scores (Table 4). After adjusting for age, sex, LDL-c, HDL-c, smoking/diabetic/hypertensive history, LogOR of methionine, homoserine and dU, all had ORs greater than 1 (MIs vs non-MIs. Figure 3I). Additionally, the ORs of succinate, IDP and glyceric acid were much less than 1, indicating they are potential protective factors for MI (Table 4).

3.5 Traditional CAD risk factors and cardiac function influence the metabolic pattern

Correlation analysis showed that methionine, homoserine, homocysteine and dU were all affected by smoking history, but none was obviously perturbed by hypertension (Table S3). However, in the subgroup analyses of smoking/nonsmoking, hypertensive/normotensive, diabetic/nondiabetic, aged 45–54/55–65 and male/female, means of homoserine, methionine and dU were higher in MI cases (Table 5).

Table 5
Mean of deoxyuridine, methionine and homoserine in certain subgroups of the controls, non-MIs and MI cases

Risk factors	Sub-groups	Deoxyuridine	Methionine	Homoserine	non-Risk factors	Sub-groups	Deoxyuridine	Methionine	Homoserine
Smokers	The controls	4.22	5.04	5.82	non Smokers	The controls	4.24	5.05	5.8
	non-MI cases	4.22	5.03	5.76		non-MI cases	4.22	5.03	5.75
	MI cases	5.48 *,#	5.44 *,#	6.13 *,#		MI cases	5.45 *,#	5.43 *,#	6.11 *,#
Diabetic	The controls	4.22	5.04	5.8	non-diabetic or insulin resistance	The controls	4.24	5.05	5.8
	non-MI cases	4.2	5.03	5.75		non-MI cases	4.21	5.04	5.73
	MI cases	5.44 *,#	5.43 *,#	6.12 *,#		MI cases	5.34 *,#	5.40 *,#	6.09 *,#
Hypertensive	The controls	4.24	5.05	5.8	Normotensive	The controls	4.22	5.04	5.8
	non-MI cases	4.22	5.03	5.75		non-MI cases	4.22	5.04	5.73
	MI cases	5.40 *,#	5.42 *,#	6.11 *,#		MI cases	5.37 *,#	5.42 *,#	6.10 *,#
Age of 45–54	The controls	4.29	5.07	5.83	Age: 55–65	The controls	4.23	5.03	5.83
	non-MI cases	4.21	5.05	5.72		non-MI cases	4.18	5.04	5.75
	MI cases	5.30 *,#	5.37 *,#	6.13*,#		MI cases	5.60 *,#	5.45 *,#	6.11 *,#
Male	The controls	4.22	5.05	5.8	Female	The controls	4.28	5.05	5.82
	non-MI cases	4.22	5.05	5.77		non-MI cases	4.23	5	5.72
	MI cases	5.43 *,#	5.44 *,#	6.11 *		MI cases	5.32 *,#	5.35 *,#	6.10 *,#

* MI vs controls, p < 0.05; #, MI vs non-MI p < 0.05.
All data were logarized before calculating, the result showed means of each subgroup.

As a clinical indicator of cardiac function in MI, positive NT-proBNP represents cardiac dysfunction. Methionine, homoserine and deoxyuridine were further elevated in NT-proBNP-positive cases. Pathway analysis of the discriminant metabolites (Table S4) between the NT-proBNP positive and negative groups suggested that only arginine biosynthesis was severely impaired (P < 0.001, FDR < 1%), indicating that arginine biosynthesis is closely associated with cardiac function. (Figure S3A)

cTnt, CK-MB, AST, LDH and HBDH are well-recognized indicators involved in myocardial damage and infarction. Six metabolites, 2-hydroxybutyrate, 3-hydroxybutyrate, homocysteine, palmitic acid, stearic acid and 1-monooleoylglycerol, were positively and significantly correlated with both cTNT and CK-MB (Figure S3C). As candidate predictors of MI, methionine, dU and homoserine were significantly and positively correlated with LDH, HBDH and AST (Figure S3B).

3.6 Altered plasma pyrimidine and methionine metabolism in MI cases

The pathway of pyrimidine metabolism was deranged in the MI cases, as shown by the dramatic changes in dU and uracil. Figure 4D shows metabolites and metabolic enzymes in the dU-related pathway. According to a circulating transcriptomics dataset (GEO accession: GSE48060), the dU-related enzymes cytidine deaminase (CDA) and uridine phosphorylase 1 (UPP1) are upregulated in MI patient plasma. According to The Human Protein Atlas,

CDA and UPP1 are highly enriched in immune cells (mainly in neutrophils and monocytes). Figure 4G shows white blood cell (WBC) counts, neutrophil (NE) counts, monocyte (MO) and lymphocyte (LY) counts and dU abundance in MI and non-MI cases. Immune cells generally increased in MI plasma. However, statistically, the correlation analysis did not detect a relationship between dU abundance and the number of any type of immune cell (including total WBCs).

Our study on transverse aortic constriction (TAC) mouse models showed that cardiac CDA mRNA expression increased as BNP and ANF mRNA levels increased (Figure S3 D-F,H). dU level is significantly higher in the proBNP-positive group than in the proBNP-negative chest pain group (Fig. 4H). We hypothesize that dU is also a potential cardiac function marker.

Figure 4A shows methionine, homocysteine and cystathionine in a panel. Inconsistent with the observed elevation of methionine, circulating transcriptomics of MI patients (GEO accession: GSE48060) showed that among the methionine abundance-related genes, 5-Methyltetrahydrofolate-Homocysteine Methyltransferase (MTR) expression decreased and Cystathionine β -synthase (CBS) increased ($p < 0.05$, Fig. 4B). The other genes involved in methionine turnover, including methionine-tRNA ligase (MetRS), MrsB, MrsA (MARS), Mat2a and Mat1a, remained statistically unchanged in plasma. The above analysis from circulating transcriptomics suggested that cells in plasma are not likely to be responsible for the marked elevation of methionine in MI patients' blood. Instead, it is more likely that damaged cardiac tissues (or other tissues) release more or utilize less free methionine. This hypothesis is supported by the fact that a transcriptomics study of a MI mouse model (LAD) revealed that in ischemic cardiac tissue, MetRS decreased significantly in MI mouse cardiac samples. (Fig. 4C; GEO accession: GSM12346)

4. Discussions

The onset of myocardial infarction is always urgent, sudden and sometimes associated with a dim prognosis. Early warning and recognition of myocardial infarction is a great challenge for clinicians and is of crucial importance to treat suspected MI patients. The early detection and intervention of MI require growing knowledge of the biology data of MI patients. In this study, we demonstrated that metabolomic data well discriminate MI cases from other non-MI chest pain cases. Some metabolites have the potential ability to differentiate and diagnose MI patients among CAD patients with the symptom of chest pain and suggest potential markers to predict the onset of MI.

4.1 Plasma metabolic features and metabolite biomarkers for the diagnosis of MI

This study identified a panel of discriminant metabolites that were also suggested as potential markers of MI in previous reports, such as taurine, methionine, leucine, isoleucine, valine, ornithine, tryptophan, citrate, and 2-ketoglutarate.[21] To our surprise, some of the metabolites showed great potential in the diagnosis of MI. Both elevated and decreased levels of metabolites were observed in the MI group. Among the differential metabolites between MI cases and non-MI cases, 10 of them in the MI group had twice the abundance as in the non-MI group (MIs/non-MIs, FC > 2); 17 metabolites had less than half the abundance as in the non-MI cases (MIs/non-MIs, FC < 0.5).

Elevated metabolites in MI cases

Based on metabolites that were elevated in the MI group, we found that methionine and cysteine metabolisms are involved in MI. In this metabolic module, methionine is a precursor of homocysteine, homoserine is utilized in the biosynthesis of methionine, and homoserine was also reported as a serum marker for cardiac disease in atherosclerosis patients with stent restenosis [22].

Although the causes of plasma metabolite alterations could be many (e.g., gut microbial metabolites), we evaluated plasma transcriptomics data because circulating substances influence plasma metabolites most directly. Transcriptomics data showed that plasma methionine-related mRNA tends to utilize methionine and homocysteine to produce more cystathionine. Yet there was not a significant change of the key genes involved in the pathway of methionine transsulfuration. Plasma Cystathionine- β -synthase (CBS) and cystathionine- γ -lyase (CTH) a similar rna level between control and MIs, indicating blood cells methionine transsulfuration pathway in mRNA level cannot explain the elevation of methionine. One explanation is hinted at by murine MI models showing decreased levels of MARS mRNA in the heart, which suggests that the damaged heart has difficulty binding methionine to tRNA. As a result, free methionine is released into the blood and detected in the circulatory system of MI patients.

The pyrimidine metabolism pathway was also deranged in the MI cases. The elevation of dU has been identified as a potential adverse factor for nucleotide pool balance and mitochondrial function in the case of mitochondrial neurogastrointestinal encephalomyopathy[23, 24]. As a metabolite in pyridine metabolism, dU has never been suggested to play a role in cardiovascular diseases before. Unlike methionine, the elevation of dU is more likely to be related to blood cells. A previous study[25] (Dataset: GSE103182) showed that STEMI (ST-elevation myocardial infarction) patients express more neutrophil-specific CDA mRNA in plasma than NSTEMI (non-ST-elevation myocardial infarction) patients. Consistent with this finding, our data showed that in the STEMI group, both dU and neutrophil counts were higher than those in the NSTEMI group (Figure S3G). It is possible that granulocyte-specific CDA is enriched in one subset of neutrophils. The subset of neutrophils varies significantly among non-MI, STEMI and NSTEMI cases. Alternatively, the elevation of CDA could be due to the heightened necrosis of granulocytes.

Decreased metabolites in MI cases

Among all the discriminant metabolites that declined in MI cases, arginine was the most noteworthy (Fig. 3C). ROC analysis showed arginine AUC equals to 0.8865 (MIs vs non-MIs). Our study showed that as cardiac damage and function worsened, these plasma arginine biosynthesis-related metabolites dropped more (Table 3). However, arginine's role as a CAD risk factor may be underestimated because arginine bioavailability, not just arginine abundance, was more significantly changed in MI patients. Another study reported that diminished global arginine bioavailability is predictive of increased CAD risk [16].

Uracil, a pyrimidine found in RNA, was also significantly decreased in MI plasma. Figure 4D and 4F show the relationship of uracil and dU in plasma pyrimidine metabolism. The panel indicates the elevated demand for plasma uracil in MI cases and the enhanced demand for damage repair after heart attack. This suggests that MI occurrence may lead to the body continues to repair itself by producing more RNA or even DNA[26].

4.2 Potential markers for predicting the occurrence of MI

Traditional risk factors

As indicated by adjusted and unadjusted OR values, traditional risk factors, such as diabetes, hypertension, and smoking, had confounding effects on the candidate metabolite biomarkers[27]. In this study, diabetes and smoking also ranked the marked risk factors for MI occurrence, but the history of hypertension was not ($OR < 1$, $p > 0.05$). As for the key substances in lipid metabolism, higher levels of TC, TG and LDLc were confirmed as risk factors for MI groups (MIs vs non-MIs, Table S5). However, higher level of LDLc failed to demonstrate itself as a "risk" to develop non-MI cardiac chest pain. (non-MIs vs the control, Table S5). Considering that routine therapy with anti-lipidemic and antihypertensive drugs lowered LDLc and blood pressure levels, medications could be responsible for some of the controversial findings.

Metabolite risk factors

The panel of discriminant metabolites did not only offer metabolic markers facilitating the diagnosis of MI, but also contributed potential markers suggesting the conceivable occurrence of MI for prediction. Previous studies reported that methionine and dU can be promoters for CAD development because their accumulation may lead to more adverse effects on humans, e.g., high levels of methionine have been identified as atherogenic[28] and metabotoxic[29] for a long time, while dU acts as a conventional antimetabolite[30]. For the first time, our data suggest that methionine and dU are high-risk markers for predicting patients about the occurrence of MI. Moreover, consistent with a previous report suggesting the relationship of dU with insulin resistance[31], our correlation analysis also suggested that dU is partially affected by diabetes (correlation analysis vs diabetes, $p < 0.05$, Pearson's $r = 0.23$), in addition to tobacco use and HDLc level.

As a precursor of homocysteine, methionine elevation is supposed to be a promoter for the development of CAD[32]. Consistent with that idea, a previous study showed that methionine in CAD cases is significantly higher than in healthy controls and is a risk factor for CAD occurrence in an unadjusted model[14]. However, after adjusting for race, sex, age, diabetes, smoking and hypertension, methionine is not as dangerous as it is in an unadjusted model [33]. Moreover, our data showed that methionine was an independent biomarker for MI. Notably, we found that the diagnostic performance of biomarker candidates for MI varied with individual characteristics, such as diabetes. When we studied individuals with a history of diabetes, methionine achieved an AUC score as high as 100% (sensitivity: 100.00%, specificity: 100.00%). For nondiabetic inpatients, the AUC score of methionine was only 73%, which is much lower than that of diabetic inpatients. Generally, this study suggests some metabolites that have the potential to facilitate the diagnosis and prediction of MI. We believe that the traditional marker (such as Tnt) and the metabolic markers (such as dU and methionine) reflect the damage of heart at different angles that are involved in confounding factors contributing to the onset of MI. In line with most studies, the odds ratio values consistently suggested that LDL cholesterol levels is the traditional common risk factor for cardiovascular disease [34]. Unexpectedly, in this study, a high level of LDLc (whether or not in an abnormal range) is not a risk for cardiac chest pain patients. Similarly, hypertension is a risk for developing CAD-related chest pain by healthy people, but not for the development of MI by chest pain patients. It greatly challenges our further research of the underlying mechanism.

5. Conclusion

Plasma deoxyuridine, homoserine, methionine, as well as a combination of homoserine, IDP and 2-ketoglutarate were suggested as markers for MI diagnosis. The changes of some metabolites abundance in diseased samples can be explained by plasma transcriptomics and could be highly associated with the blood cells. Hopefully, the growing plasma single-cell RNA sequencing evidence [35, 36] and omics data (e.g., cfDNA methylome[37]) offer a clue to reveal the mechanism behind the changes soon.

6. Abbreviations

MI, myocardial infarction; CAD, coronary artery disease; UA, unstable angina; non-MIs, non-MI chest pain cases; ECG, Electrocardiogram; ROC, receiver operating characteristic curve; AUC, area under the ROC Curve; QC, quality control; IS, internal standard; VIP, variable Importance; QC, quality control, IS, internal standard, PCA, Principal component analysis, PLS-DA, partial least square to latent structure discriminant analysis; OPLS-DA, orthogonal PLS-DA; OR, odds ratios; dU, deoxyuridine; TAC, transverse aortic constriction; LAD, left anterior descending coronary artery; CDA, cytidine deaminase; UPP1, uridine phosphorylase 1; MARS, methionyl-tRNA synthetase; WBC, white blood cell counts; NE, neutrophil; MO, monocyte and LY, lymphocyte.

7. Declarations

7.1. Ethics approval and consent to participate

The study followed the principles outlined in the Declaration of Helsinki, informed written consent was given prior to the inclusion of subjects in the study. The study was also under the guidance and supervision of Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Lot number: 2018-SR-028).

7.2. Consent for publication

Not applicable.

7.3. Availability of data and materials

The raw data analyzed in the study are available from the corresponding author on reasonable request.

7.4. Competing interests

The authors declare that they have no competing interests.

7.5. Funding

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7.6. Authors' contributions

Prof. Xiangqing Kong, Jiye Aa came up with the idea of the study; Prof. Guangji Wang and Jiye Aa provided the LC/MS and GC/MS platform. Dr. Zhijian Yang, Liansheng Wang and Chunjian Li confirmed the diagnosis of MI, non-MI chest pain cases. Ms. Nan Aa, Ying Lu and Heng Tang collected the blood samples, recorded the medical history of the volunteers and prepared the plasma samples. Ms. Nan Aa, Mr. Zhenyao Lu and Dr. Runbing Sun performed the untargeted metabolomics. Ms. Nan Aa and Mr. Mengjie Yu analyzed the data. Figures and tables were produced by Ms. Nan Aa, Mr. Mengjie Yu and Prof. Jiye Aa. Prof. Xiangqing Kong, Ms. Nan Aa and Ms. Ying Lu wrote the manuscript.

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References

1. Hammarsten O, Mair J, Mockel M, Lindahl B, Jaffe AS. Possible mechanisms behind cardiac troponin elevations. *Biomarkers*. 2018;23:725–34.
2. Nowak R, Mueller C, Giannitsis E, Christ M, Ordonez-Llanos J, DeFilippi C, McCord J, Body R, Panteghini M, Jernberg T, Plebani M, Verschuren F, French JK, Christenson R, Jacobsen G, Dinkel C, Lindahl B. High sensitivity cardiac troponin T in patients not having an acute coronary syndrome: results from the TRAPID-AMI study. *Biomarkers*. 2017;22:709–14.
3. Riedlinger D, Mockel M, Muller C, Holert F, Searle J, von Recum J, Slagman A. High-sensitivity cardiac troponin T for diagnosis of NSTEMI in the elderly emergency department patient: a clinical cohort study. *Biomarkers*. 2018;23:551–7.
4. Lopaschuk GD. Metabolic Modulators in Heart Disease: Past, Present, and Future. *Can J Cardiol*. 2017;33:838–49.
5. Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HW, Clarke S, Schofield PM, McKilligin E, Mosedale DE, Grainger DJ. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using ¹H-NMR-based metabolomics. *Nat Med*. 2002;8:1439–44.
6. Sabatine MS, Liu E, Morrow DA, Heller E, McCarroll R, Wiegand R, Berriz GF, Roth FP, Gerszten RE. Metabolomic identification of novel biomarkers of myocardial ischemia. *Circulation*. 2005;112:3868–75.
7. Shah SH, Kraus WE, Newgard CB. Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. *Circulation*. 2012;126:1110–20.
8. Lewis GD, Asnani A, Gerszten RE. Application of metabolomics to cardiovascular biomarker and pathway discovery. *J Am Coll Cardiol*. 2008;52:117–23.

9. Fan Y, Li Y, Chen Y, Zhao YJ, Liu LW, Li J, Wang SL, Alolga RN, Yin Y, Wang XM, Zhao DS, Shen JH, Meng FQ, Zhou X, Xu H, He GP, Lai MD, Li P, Zhu W, Qi LW. Comprehensive Metabolomic Characterization of Coronary Artery Diseases. *J Am Coll Cardiol*. 2016;68:1281–93.
10. Cheng ML, Wang CH, Shiao MS, Liu MH, Huang YY, Huang CY, Mao CT, Lin JF, Ho HY, Yang NI. Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: diagnostic and prognostic value of metabolomics. *J Am Coll Cardiol*. 2015;65:1509–20.
11. Stegemann C, Pechlaner R, Willeit P, Langley SR, Mangino M, Mayr U, Menni C, Moayyeri A, Santer P, Rungger G, Spector TD, Willeit J, Kiechl S, Mayr M. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based Bruneck study. *Circulation*. 2014;129:1821–31.
12. Zhang L, Wei TT, Li Y, Li J, Fan Y, Huang FQ, Cai YY, Ma G, Liu JF, Chen QQ, Wang SL, Li H, Alolga RN, Liu B, Zhao DS, Shen JH, Wang XM, Zhu W, Li P, Qi LW, Functional Metabolomics Characterizes a Key Role for N-Acetylneuraminic Acid in Coronary Artery Diseases, *Circulation*, 2018, 137: 1374–1390.
13. Song JP, Chen L, Chen X, Ren J, Zhang NN, Tirasawasdichai T, Hu ZL, Hua W, Hu YR, Tang HR, Chen HV, Hu SS. Elevated plasma beta-hydroxybutyrate predicts adverse outcomes and disease progression in patients with arrhythmogenic cardiomyopathy, *Sci Transl Med*, 2020, 12.
14. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ*. 2002;325:1202.
15. Floegel A, Kuhn T, Sookthai D, Johnson T, Prehn C, Rolle-Kampczyk U, Otto W, Weikert C, Illig T, von Bergen M, Adamski J, Boeing H, Kaaks R, Pischon T. Serum metabolites and risk of myocardial infarction and ischemic stroke: a targeted metabolomic approach in two German prospective cohorts. *Eur J Epidemiol*. 2018;33:55–66.
16. Tang WH, Wang Z, Cho L, Brennan DM, Hazen SL. Diminished global arginine bioavailability and increased arginine catabolism as metabolic profile of increased cardiovascular risk. *J Am Coll Cardiol*. 2009;53:2061–7.
17. Grund B, Sabin C. Analysis of biomarker data: logs, odds ratios, and receiver operating characteristic curves. *Curr Opin HIV AIDS*. 2010;5:473–9.
18. Trygg JA, J, Gullberg J, Johansson AI, Jonsson P, Antti H, Marklund SL, Moritz T. Extraction and GC/MS analysis of the human blood plasma metabolome. *Anal Chem*. 2005;77:8086–94.
19. Aa N, Guo JH, Cao B, Sun RB, Ma XH, Chu Y, Zhou SP, Aa JY, Yang ZJ, Sun H, Wang GJ. Compound danshen dripping pills normalize a reprogrammed metabolism of myocardial ischemia rats to interpret its time-dependent efficacy in clinic trials: a metabolomic study. *Metabolomics*. 2019;15:128.
20. Zhang Y, Lu W, Wang Z, Zhang R, Xie Y, Guo S, Jiao L, Hong Y, Di Z, Wang G, Aa J. Reduced Neuronal cAMP in the Nucleus Accumbens Damages Blood-Brain Barrier Integrity and Promotes Stress Vulnerability. *Biol Psychiatry*. 2020;87:526–37.
21. Sansbury BE, DeMartino AM, Xie Z, Brooks AC, Brainard RE, Watson LJ, DeFilippis AP, Cummins TD, Harbeson MA, Brittian KR, Prabhu SD, Bhatnagar A, Jones SP, Hill BG. Metabolomic analysis of pressure-overloaded and infarcted mouse hearts. *Circ Heart Fail*. 2014;7:634–42.
22. Hasokawa M, Shinohara M, Tsugawa H, Bamba T, Fukusaki E, Nishiumi S, Nishimura K, Yoshida M, Ishida T, Hirata K. Identification of biomarkers of stent restenosis with serum metabolomic profiling using gas chromatography/mass spectrometry. *Circ J*. 2012;76:1864–73.
23. Bax BE, Levene M, Bain MD, Fairbanks LD, Filosto M, Kalkan Ucar S, Klopstock T, Kornblum C, Mandel H, Rahman S, Roubertie A, Scarpelli M, Sedgwick PM, Baru M, Sello-Moura M, Price J, Horn P, Nirmalanathan N. Erythrocyte Encapsulated Thymidine Phosphorylase for the Treatment of Patients with Mitochondrial Neurogastrointestinal Encephalomyopathy: Study Protocol for a Multi-Centre, Multiple Dose, Open Label Trial, *J Clin Med*, 2019, 8.
24. Marti R, Nishigaki Y, Hirano M. Elevated plasma deoxyuridine in patients with thymidine phosphorylase deficiency. *Biochem Biophys Res Commun*. 2003;303:14–8.
25. Chiesa M, Piacentini L, Bono E, Milazzo V, Campodonico J, Marenzi G, Colombo GI. Whole blood transcriptome profile at hospital admission discriminates between patients with ST-segment elevation and non-ST-segment elevation acute myocardial infarction. *Sci Rep*. 2020;10:8731.
26. Krokan HE, Drablos F, Slupphaug G. Uracil in DNA—occurrence, consequences and repair. *Oncogene*. 2002;21:8935–48.
27. Foody J, Huo Y, Ji L, Zhao D, Boyd D, Meng HJ, Shiff S, Hu D. Unique and Varied Contributions of Traditional CVD Risk Factors: A Systematic Literature Review of CAD Risk Factors in China. *Clin Med Insights Cardiol*. 2013;7:59–86.
28. Troen AM, Lutgens E, Smith DE, Rosenberg IH, Selhub J. The atherogenic effect of excess methionine intake. *Proc Natl Acad Sci U S A*. 2003;100:15089–94.
29. Murphy-Chutorian DR, Wexman MP, Grieco AJ, Heininger JA, Glassman E, Gaull GE, Ng SK, Feit F, Wexman K, Fox AC. Methionine intolerance: a possible risk factor for coronary artery disease. *J Am Coll Cardiol*. 1985;6:725–30.
30. Das KC, Herbert V. The lymphocyte as a marker of past nutritional status: persistence of abnormal lymphocyte deoxyuridine (dU) suppression test and chromosomes in patients with past deficiency of folate and vitamin B12. *Br J Haematol*. 1978;38:219–33.
31. Li LO, Hu YF, Wang L, Mitchell M, Berger A, Coleman RA. Early hepatic insulin resistance in mice: a metabolomics analysis. *Mol Endocrinol*. 2010;24:657–66.
32. Kim J, Kim H, Roh H, Kwon Y. Causes of hyperhomocysteinemia and its pathological significance. *Arch Pharm Res*. 2018;41:372–83.
33. Shah SH, Bain JR, Muehlbauer MJ, Stevens RD, Crosslin DR, Haynes C, Dungan J, Newby LK, Hauser ER, Ginsburg GS, Newgard CB, Kraus WE. Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. *Circ Cardiovasc Genet*. 2010;3:207–14.
34. Holmes MV, Millwood IY, Kartsonaki C, Hill MR, Bennett DA, Boxall R, Guo Y, Xu X, Bian Z, Hu R, Walters RG, Chen J, Ala-Korpela M, Parish S, Clarke RJ, Peto R, Collins R, Li L, Chen Z. G. China Kadoorie Biobank Collaborative, Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction

35. Abplanalp WT, John D, Cremer S, Assmus B, Dorsheimer L, Hoffmann J, Becker-Pergola G, Rieger MA, Zeiher AM, Vasa-Nicotera M, Dimmeler S. Single cell RNA sequencing reveals profound changes in circulating immune cells in patients with heart failure, Cardiovasc Res, 2020.
36. Ren H, Liu X, Wang L, Gao Y. Lymphocyte-to-Monocyte Ratio: A Novel Predictor of the Prognosis of Acute Ischemic Stroke. J Stroke Cerebrovasc Dis. 2017;26:2595–602.
37. Zemmour H, Planer D, Magenheimer J, Moss J, Neiman D, Gilon D, Korach A, Glaser B, Shemer R, Landesberg G, Dor Y. Non-invasive detection of human cardiomyocyte death using methylation patterns of circulating DNA. Nat Commun. 2018;9:1443.

Figures

Figure 1

Multivariate statistical analysis differentiates the groups of MI cases, non-MI chest pain cases and the controls based on clinical information(A) and metabolomic data(B), respectively. 1. Non-supervised PCA modeling displays the original similarity of three groups of MI cases(n=85), non-MI chest pain cases(n=61) and the controls(n=84), without arbitrary grouping. 2. Non-supervised PCA modeling displays the original similarity of four groups of MI cases, UA, other non-MI cardiac cases and the controls, without arbitrary grouping. A1&A2, PC1:R2X(cum)=0.0895, Q2(cum)=0.0106; PC2:R2X(cum)=0.167, Q2(cum)=0.0356. B1&B2, PC1:R2X(cum)=0.205, Q2(cum)=0.189; PC2:R2X(cum)=0.310, Q2(cum)=0.274, PC3:R2X(cum)=0.386, Q2(cum)=0.338. 3. Supervised PLS-DA modeling with three groups of MI cases, non-MI chest pain cases and the controls. A3, PC1:R2X(cum)=0.073, R2Y(cum)=0.339, Q2(cum)=0.270; PC2:R2X(cum)=0.128, R2Y(cum)=0.544, Q2(cum)=0.362; PC3:R2X(cum)=0.174, R2Y(cum)=0.635, Q2(cum)=0.394. Permutation tests with the intercepts of R2<0.23, Q2<0.20. B3, PC1:R2X(cum)=0.184, R2Y(cum)=0.215, Q2(cum)=0.207; PC2:R2X(cum)=0.249, R2Y(cum)=0.400, Q2(cum)=0.377, PC3:R2X(cum)=0.315, R2Y(cum)=0.547, Q2(cum)=0.512. Permutation tests with the intercepts of R2<0.10, Q2<0.05. 4. Supervised PLS-DA modeling with four groups of MI cases(n=85), UA(n=34), other non-MI cardiac cases(n=27) and the controls(n=84). A4, PC1:R2X(cum)=0.073, R2Y(cum)=0.259, Q2(cum)=0.198; PC2:R2X(cum)=0.132, R2Y(cum)=0.397, Q2(cum)=0.269; PC3:R2X(cum)=0.173, R2Y(cum)=0.492, Q2(cum)=0.289. Permutation test with the intercepts of R2<0.180, Q2<0.15. B4,PC1:R2X(cum)=0.183, R2Y(cum)=0.169,

Q2(cum)=0.163; PC2:R2X(cum)=0.254, R2Y(cum)=0.300, Q2(cum)=0.277, PC3:R2X(cum)=0.317, R2Y(cum)=0.422, Q2(cum)=0.387 . Permutation test with the intercept of R2<0.11, Q2<0.05.

Figure 2

OPLS-DA modeling and S-plots delineate different metabolic phenotypes and potential markers of MI and non-MI cardiac cases. A1, OPLS-DA model differentiating non-MI cardiac cases(n=61) from the controls(n=84); A2, S-plot highlights the potential markers of the non-MI cardiac cases. B1, OPLS-DA model differentiating MI cases(n=85) from non-MI cardiac cases(n=61); B2, S-plot highlights the potential markers of the MI cases.

Figure 3

Differential metabolites and pathways involved in MI group A) Samples are divided into 3 groups: control(n=84), MI(n=85) cases and non-MI chest pain cases(n=61) or 4 groups: control(n=84), MI cases(n=85), UA(n=34) and the other non-MI cardiac cases(n=27) when OPLS-DA analysis is applied. B) Plasma methionine, deoxyuridine and homoserine levels spike in MI group. C) Succinate, inosine and arginine levels drop as CAD progresses to MI. D) Gluconic acid and isoleucine levels increase as CAD progresses to MI. E) Venn diagram shows discriminant metabolites can be classified into region A,B and C. F) Pathway analysis of differential metabolites in Venn A&B region (control vs non-MI chest pain cases) G) Pathway analysis of differential metabolites in Venn C&B (MI vs non-MI cases). There is remarkable change of arginine biosynthesis and alanine,aspartate, glutamate metabolism. . H) Pathway analysis of differential metabolites in Venn C. There is remarkable change of arginine biosynthesis and alanine,aspartate, glutamate metabolism. . I) Unadjusted and adjusted (adjusted for age, gender, LDL-c, HDL-c, smoking/diabetic/hypertensive history) odd ratio of methionine, deoxyuridine and homoserine. J) ROC analysis of homoserine (AUC 94.31%, sensitivity 80.9%, specificity 100%), methionine(AUC 96.43%, sensitivity 89.4%, specificity 94.59%), homocysteine(AUC 72.78%); K) ROC analysis of deoxyuridine (AUC 91.66%, sensitivity 80.9%, specificity 100%); L) ROC analysis of a combination of homoserine, IDP and 2-ketoglutarate (AUC=97%, sensitivity 94.12%, specificity 100%).