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The interactions and biological pathways among metabolomics products of patients with coronary heart disease

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ABSTRACT

Background: Through bioinformatics analysis, this study explores the interactions and biological pathways involving metabolomic products in patients diagnosed with coronary heart disease (CHD).
Methods: A comprehensive search for relevant studies focusing on metabolomics analysis in CHD patients was conducted across databases including CNKI, Wanfang, VIP, CBM, PubMed, Cochrane Library, Nature, Web of Science, Springer, and Science Direct. Metabolites reported in the literature underwent statistical analysis and summarization, with the identification of differential metabolites. The pathways associated with these metabolites were examined using the Kyoto Encyclopedia of Genes and Genomes (KEGG). Molecular annotation of metabolites and their relationships with enzymes or transporters were elucidated through analysis with the Human Metabolome Database (HMDB). Visual representation of the properties related to these metabolites was achieved using Metabolomics Pathway Analysis (metPA).
Results: A total of 13 literatures satisfying the criteria for enrollment were included. A total of 91 metabolites related to CHD were preliminarily screened, and 87 effective metabolites were obtained after the unrecognized metabolites were excluded. A total of 45 pathways were involved. Through the topology analysis (TPA) of pathways, their influence values were calculated, and 13 major metabolic pathways were selected. The pathways such as Phenylalanine, tyrosine, and tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, and Glycine, serine, and threonine metabolism primarily involved the regulation of processes and metabolites related to inflammation, oxidative stress, one-carbon metabolism, energy metabolism, lipid metabolism, immune regulation, and nitric oxide expression.
Conclusion: Multiple pathways, including Phenylalanine, tyrosine, and tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, and Glycine, serine, and threonine metabolism, were involved in the occurrence of CHD. The occurrence of CHD is primarily associated with the regulation of processes and metabolites related to inflammation, oxidative stress, one-carbon metabolism, energy metabolism, lipid metabolism, immune regulation, and nitric oxide expression.

1. Introduction

Coronary heart disease (CHD) is a chronic condition characterized by the stenosis or occlusion of coronary arteries due to atherosclerosis, resulting in myocardial ischemia, hypoxia, or necrosis. With a notable prevalence in economically developed countries, CHD stands as the leading cause of cardiovascular-related mortality globally, contributing to approximately 8.9 million deaths annually [1]. The rapid economic development in China has propelled CHD to become one of the top three causes of death in the country, and its onset is increasingly observed at a younger age [2]. Recent statistical data indicates a discernible upward trend in CHD mortality rates in both urban and rural areas, with a higher incidence observed in urban settings [3]. CHD manifests with diverse symptoms, including asymptomatic myocardial ischemia, angina pectoris, ischemic cardiomyopathy, and myocardial infarction. Despite extensive epidemiological and clinical investigations, the etiology of CHD remains elusive. Numerous studies highlight the association of CHD with factors such as age, gender, dyslipidemia, hypertension,

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smoking, obesity, and family history [4,5]. Currently, the clinical diagnosis of CHD predominantly relies on typical clinical symptoms, complemented by imaging auxiliary examination methods including ultrasonography, electrocardiogram, coronary angiography, and echocardiography. Despite their utility, these diagnostic approaches exhibit drawbacks such as low safety or sensitivity, high cost, and elevated risk [6]. Clinical treatment for CHD patients primarily involves drug therapy, interventional procedures, and surgical interventions. However, these modalities have inherent limitations, with the therapeutic effectiveness falling short of achieving a 100% success rate. Additionally, there are notable dependencies on therapy and associated drug side effects [7]. Consequently, the exploration of novel diagnostic and treatment methodologies for CHD holds significant importance.

Metabolic abnormalities constitute a primary clinical feature of CHD, and there exists a significant correlation between the severity of CHD and metabolic irregularities [8]. Metabolomics, also known as metabonomics or metabolomics, is a discipline that investigates living organisms by examining the types, quantities, and patterns of changes in their metabolites in response to various stimuli or disturbances. It represents a novel approach to studying the metabolic pathways of biological systems. Metabolomics employs techniques such as nuclear magnetic resonance (NMR), liquid chromatograph mass spectrometer (LC-MS), gas chromatography-mass spectrometry (GC-MS), and other detection methods. Taking an omics perspective, it reveals alterations in the entire metabolic network pathway within a biological organism, offering insights into a series of biological events occurring in specific physiological and pathological states of the organism. Metabolomics analysis technology is distinguished by its high throughput, sensitivity, and accuracy, enabling the identification of endogenous metabolites that align with physiological and pathological changes in diseases [9]. In recent years, metabolomics has found widespread application in elucidating the pathogenesis of various diseases, developing disease diagnosis and treatment strategies, conducting gene analysis, screening biomarkers, and facilitating drug design and development [10]. While scholars have engaged in discussions on metabolism-related studies in metabolomics of CHD, variations in research techniques, subjects, and control conditions have led to discrepancies in CHD metabolites [11]. Additionally, the pathogenesis of CHD involves a complex process with multiple factors and layers, and existing studies have not fully clarified the interaction between metabolites and biological pathway information [12].

Building upon the existing studies on metabolomics analysis of CHD patients, this study consolidated relevant data from various sources, including different studies and metabolites. Subsequently, a bioinformatics analysis was conducted to understand the biological functions of these metabolites. Furthermore, the correlation between the occurrence of CHD and potential differentially regulated metabolites was investigated. Pathway analysis was then performed on these differential metabolites to unveil the molecular-level pathogenesis of CHD. This comprehensive approach aimed to contribute to the theoretical foundation for the diagnosis and treatment of CHD.

2. Materials and methods

2.1. The methods to retrieve the studies

Related studies on the metabolomics analysis of CHD were retrieved from various online databases, including CNKI, Wanfang, VIP, CBM, PubMed, Cochrane Library, Nature, Web of Science, Springer, and Science Direct.

(1) Search time and language: the search period extended from the establishment of each database to October 31, 2023, with no language restrictions. (2) Search keywords: the search utilized keywords such as coronary artery heart disease, CHD, coronary artery disease, acute coronary artery heart, coronary artery disease, “CAD,” metabolomics, and Metabolome. (3) Retrieval strategy: the search involved combining subject terms with their related extension terms in the specified online databases. Logical operation symbols “or” and “and” were used for joint searches between subject terms and extension terms. Additionally, reference catalogues of relevant reviews were scanned, and any potentially missed studies were manually searched for.

2.2. Criteria for determining the enrollment or not of the studies

The criteria for selecting relevant articles were as follows: (1) the subjects were CHD patients; (2) metabolites of CHD patients were analyzed using metabonomics; (3) there was a control group comprising non-CHD healthy subjects; (4) the studies were randomized controlled trials (RCTs); and (5) differences in metabolites between CHD patients and the control group were analyzed in detail.

The criteria for excluding articles were as follows: (1) reviews, individual case reports, response books, and editorials; (2) literature with animals as research subjects; (3) documents with original data that could not be obtained through various methods and approaches; (4) repeated publication of literature; and (5) studies on non-metabolomic small molecule compounds related to CHD.

2.3. Identification of compounds in metabolomics

According to the criteria outlined in the previous section, relevant studies on CHD metabolites were included, and the metabolites were statistically analyzed and summarized based on the included literature. Repeated metabolite statistics were counted only once. Names that were uncertain or unclear in the product were temporarily excluded from the count. The metPA website’s conversion tool (https://www.metaboanalyst.ca/) was utilized for standardizing various metabolite names.

For individual genes whose metPA could not be identified, a manual search was conducted through the Human Metabolome Database (HMDB), PubChem, and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to obtain their standardized names.

2.4. Bioinformatics analysis of CHD metabolism

The chosen differential metabolites underwent analysis utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/keg/) pathway. Molecular annotation of the metabolites, along with the examination of related enzymes or transporters and their associated properties, was conducted using HMDB (http://www.hmdb.ca/). Furthermore, the visualization of metabolite pathways was performed through metPA.

3. Results

3.1. Analysis of retrieval results

A total of 119 articles were identified through keyword searches in the aforementioned online databases. Upon preliminary screening, 67 reviews, individual case reports, and animal studies were excluded, resulting in 52 articles being provisionally retained. Subsequently, through a detailed examination of the titles and abstracts, 34 articles related to the metabonomics analysis of non-CHD patients were excluded, leaving 18 articles for further assessment based on their content. Five studies that either lacked elaboration on the specific differential metabolites or were unable to provide raw data for these metabolites were excluded. Finally, 13 articles met the inclusion criteria for this study. The detailed process is depicted in Fig. 1.

3.2. Analysis of metabolomics characteristics mentioned in the enrolled literature

The metabolomic characteristics of the included studies are presented in Table 1. A total of 13 studies [13–25] were ultimately included in this analysis. With the exception of two studies, urine samples were

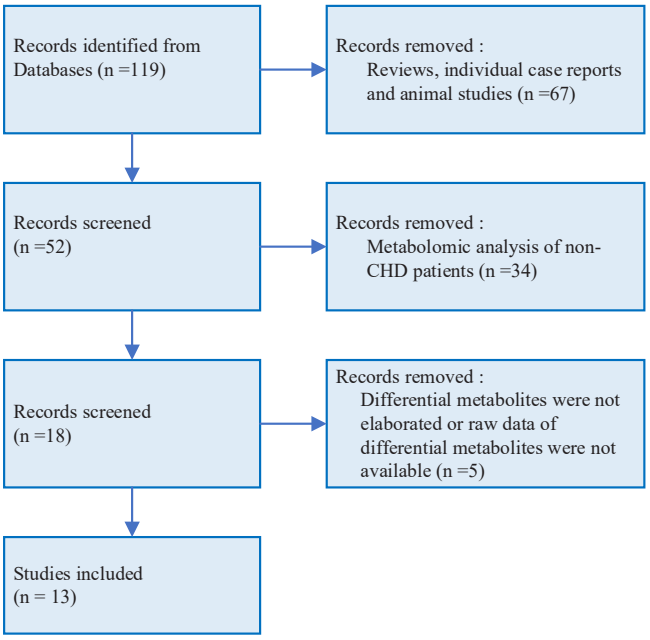

Fig. 1. The process to retrieve the required literatures.

utilized, while all other studies employed blood samples. The cumulative analysis involved a total of 6291 patients, comprising 2341 CHD patients and 3950 control (Ctrl) patients. Upon consolidating the metabolite data from various studies, a total of 91 metabolites were identified to be associated with the occurrence of CHD.

3.3. Analysis of retrieval results of differential metabolites using HMDB

The metPA Web conversion tool and HMDB were utilized to standardize metabolite names. Subsequently, a total of 87 metabolites were obtained, excluding any unrecognized metabolites. The outcomes of the HMDB retrieval process are presented in Table 2.

3.4. TPA of metabolic pathways in patients with CHD

A total of 87 metabolomic entities were implicated across 45 pathways in patients with CHD. The influence values of the TPA pathway were computed, and the outcomes of the 45 metabolic pathways involving 87 metabolites are depicted in Fig. 2. It is evident from the illustration that these metabolites predominantly engage in the regulation of pathways such as Phenylalanine, Tyrosine, and Tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, Glycine, Serine, and Threonine metabolism, Biosynthesis of unsaturated fatty acids, Arginine and Proline metabolism, Phenylalanine metabolism, Alanine, Aspartate, and Glutamate metabolism, Glycerophospholipid metabolism, Linoleic acid metabolism, Arginine biosynthesis, Butanoate metabolism, Pyruvate metabolism, Glycolysis/Gluconeogenesis, Galactose metabolism, Neomycin, kanamycin, and gentamicin biosynthesis, Arachidonic acid metabolism, Lipoic acid metabolism, Glutathione metabolism, and Alpha-Linolenic acid metabolism.

Following topological analysis and impact value calculation, 13 metabolic pathways exhibiting a significance level of $P<0.05$ were discerned as potentially linked to CHD. Detailed statistical information is provided in Table 3. Notably, the 13 pathways demonstrating a significant correlation with CHD occurrence encompass Phenylalanine, Tyrosine, and Tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, Glycine, Serine, and Threonine metabolism, Biosynthesis of unsaturated fatty acids, Arginine and

Table 1
Statistical of metabolomics features.

The first author	Year	Sample	Detection method	Sample size	CHD group (cases)	Ctrl group (cases)	The major metabolites
Jian WX [13]	2010	Urine	GC-MS	6	3	3	Glycerol, oleamide, UA, xylitol
Yi M [14]	2021	Plasma	1 H NMR	109	69	40	3-hydroxybutyric acid, alanine, lactic acid, glutamic acid, phosphatidylcholine, glycine, glycerol phosphatidylcholine, Phe, citric acid, formate
Wu G [15]	2021	Serum	LC-MS/MS	67	37	30	DL-O-phosphoserine, L-Phe, isocitric acid, glycine, acetic acid, dimethylglycine, OA, pyroglutamic acid, L-glutamic acid, L-leucine, LOA, phenylpyruvic acid, oxoglutaric acid, phosphatidylcholine
Li R [16]	2016	Plasma	LC-MS	83	40	43	2-Naphthol, N-Acetyl-D-glucosamine 6-phosphate, 1-Naphthol, and L-Carnitine
Paynter NP [17]	2018	Plasma	LC-MS	944	472	472	5-HETE, 15-HETE, 11- HETE, asparagine, glutamate, cytidine monophosphate, and diacylglycerol
Guo N [18]	2021	Serum	LC-MS	60	30	30	Histidine, UA, hypoxanthine, arachidonic acid, LOA, citric acid, lysophosphatidylcholine
Zhao LL [19]	2019	Plasma	1 H NMR	53	27	26	Lipid, taurine, α-glucose, choline, tyrosine, Phe
Huang M [20]	2019	Urine	UPLC-Q-TOF/MS	160	131	29	4-Hydroxyproline, 5-Hydroxyindoleacetaldehyde, 2-Isopropylmalic acid, Adenosine, Isobutyryl-carnitine, Leucyl-Phe, Indole-3-carboxylic acid, 4-hydroxyhippuric acid, isoleucylproline, adipic acid, N-Acetyl-b-D-galactosamine, Prolylhydroxyproline, Nicotinuric acid
Zhong Z [21]	2019	Plasma	LC-MS	361	302	59	Choline, creatinine, carnitine
Ullah E [22]	2022	Plasma	UPLC-MS/MS	4000	1001	2999	Ornithine, 3-Amino-2-piperidone, glutamate, pyruvate, sphingosine 1-phosphate, linoleoyl carnitine, oleoylcarnitine, IDA
Li Y [23]	2017	Serum	UHPLC-QTOF/MS	300	150	150	PMA, linoleic acid, 4-pyridoxic acid, PG, LCA
Feng Q [24]	2016	Plasma	LC-MS	102	59	43	L-Arginine, paraxanthine, and lysophosphatidylcholine
Hu F [25]	2023	Serum	GC-MS	46	20	26	Docosahexaenoic acid, Zymosterol, Lathosterol, Isocitric acid, L-cystine, D-tagatose, D-fructose, Glycerol 3-phosphate, Arachidonic acid, Glycocyamine, Pyrophosphate, LCA, Phytosphingosine, Agmatine, Nicotinic acid, Succinic acid, Dehydroascorbic acid

Note: UA: uric acid; OA: oleic acid; LOA: alpha-linolenic acid; LCA: lithocholic acid; PG: phosphatidylglycerol; IDA: lminodiacetate; HETE: hydroxyeicosatetraenoic acid; PMA: Palmitic acid.

Table 2
Bioinformatics notes of metabolome products in CHD patients.

Metabolites	HMDB ID	PubChem	KEGG ID
Glycerol	HMDB0000131	753	C00116
oleamide	HMDB0002117	5283387	C19670
xylitol	HMDB0002917	6912	C00379
3-hydroxybutyric acid	HMDB0000011	92135	C01089
alanine	-	-	C01401
lactic acid	HMDB0000190	107689	C00186
glutamic acid	HMDB0000148	33032	C00302
glycerol phosphatidylcholine	METPA0476	NA	C04233
Phe	HMDB0000159	6140	C00079
formate	HMDB0000142	284	C00058
DL-O-phosphoserine	HMDB0001721	106	C01005
glycine	HMDB0000123	750	C00037
acetic acid	HMDB0000042	176	C00033
dimethylglycine	HMDB0000092	673	C01026
oleic acid	HMDB0000207	445639	C00712
pyroglutamic acid	HMDB0000267	7405	C01879
L-glutamic acid	HMDB0000148	33032	C00302
L-leucine	HMDB0000687	6106	C00123
alpha-linolenic acid	HMDB0001388	5280934	C06427
phenylpyruvic acid	HMDB0000205	997	C00166
oxoglutaric acid	HMDB0000208	51	C00026
phosphatidylcholine	-	-	C00157
2-Naphthol	HMDB0012322	8663	C11713
N-Acetyl-D-glucosamine 6-phosphate	HMDB0001062	440996	C00357
1-Naphthol	HMDB0012138	7005	C11714
L-Carnitine	HMDB0000062	10917	C00487
5-hydroxyeicosatetraenoic acid	HMDB0011134	5280733	C04805
15-hydroxyeicosatetraenoic acid	HMDB0003876	5280724	C04742
11-hydroxyeicosatetraenoic acid	HMDB0004682	5312981	C14780
Asparagine	HMDB0000168	6267	C00152
cytidine monophosphate	HMDB0000095	6131	C00055
Histidine	HMDB0000177	6274	C00135
Uric acid	HMDB0000289	1175	C00366
hypoxanthine	HMDB0000157	790	C00262
linolenic acid	HMDB0001388	5280934	C06427
citric acid	HMDB0000094	311	C00158
taurine	HMDB0000251	1123	C00245
α-glucose	HMDB0000122	64689	C00031
tyrosine	HMDB0000158	6057	C00082
phenylalanine	HMDB0000159	6140	C00079
5-Hydroxyindoleacetaldehyde	HMDB0004073	74688	C05634
2-Isopropylmalic acid	HMDB0000402	5280523	C02504
4-Hydroxyproline	HMDB0000725	5810	C01157
Adenosine	HMDB0000050	60961	C00212
Isobutyryl-carnitine	HMDB0000736	168379	-
Leucyl-phenylalanine	HMDB0013243	6992309	C11221
Indole-3-carboxylic acid	HMDB0003320	69867	C19837
4-hydroxyhippuric acid	HMDB0013678	151012	-
Isoleucylproline	HMDB0011174	444876	-
Adipic acid	HMDB0000448	196	C06104
N-Acetyl-b-D-galactosamine	HMDB0000853	440552	C05021
Prolylhydroxyproline	HMDB0006695	11902892	-
Nicotinuric acid	HMDB0003269	68499	C05380
Choline	HMDB0000097	305	C00114
creatinine	HMDB0000562	588	C00791
carnitine	HMDB0000062	10917	C00487
Ornithine	HMDB0000214	6262	C00077
3-Amino-2-piperidone	HMDB0000323	5200225	-
Glutamate	HMDB0000148	33032	C00302
Pyruvate	HMDB0000243	1060	C00022
Sphingosine 1-phosphate	HMDB0000277	5283560	C06124
Linoleoyl carnitine	HMDB0006469	6450015	-
Oleoylcarnitine	HMDB0005065	46907933	-
lminodiacetate	HMDB0011753	8897	C19911
palmitic acid	HMDB0000220	985	C00249
linoleic acid	HMDB0000673	5280450	C01595
4-pyridoxic acid	HMDB0000017	6723	C00847
phosphatidylglycerol	HMDB0302468	45109789	C00344
L-Arginine	HMDB0000517	6322	C00062
Paraxanthine	HMDB0001860	4687	C13747
lithocholic acid	HMDB0000761	9903	C03990
Docosahexaenoic acid	HMDB0002183	445580	C06429
Zymosterol	HMDB0006271	92746	C05437
Lathosterol	HMDB0001170	65728	C01189

Table 2 (*continued*)

Metabolites	HMDB ID	PubChem	KEGG ID
Isocitric acid	HMDB0000193	1198	C00311
L-cystine	HMDB0000192	67678	C00491
D-tagatose	HMDB0003418	14408225	C00795
D-fructose	HMDB0000660	439709	C00095
Glycerol 3-phosphate	HMDB0000126	439162	C00093
Arachidonic acid	HMDB0001043	444899	C00219
Glycocyamine	HMDB0000128	763	C00581
Pyrophosphate	HMDB0000250	1023	C00013
Phytosphingosine	HMDB0004610	122121	C12144
Agmatine	HMDB0001432	199	C00179
Nicotinic acid	HMDB0001488	938	C00253
Succinic acid	HMDB0000254	1738118	C00042
Dehydroascorbic acid	HMDB0001264	440667	C05422

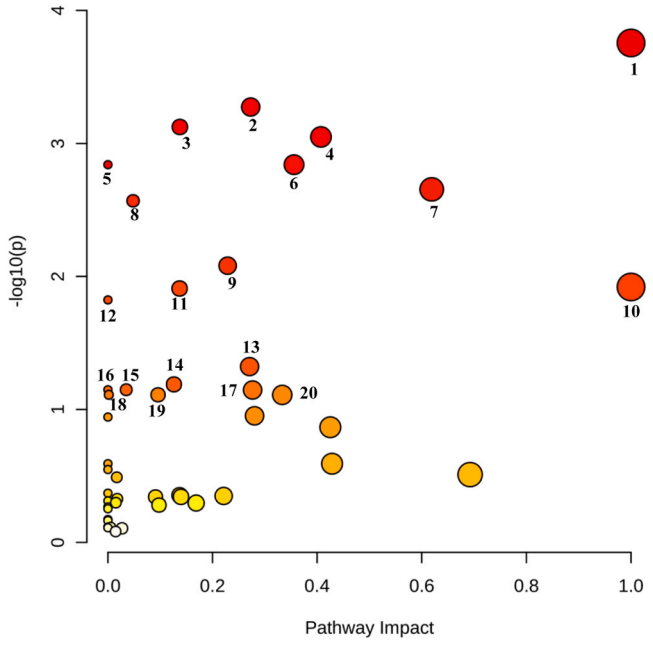

Fig. 2. Summary of MetPA analysis of metabolites pathway for patients. (1: phenylalanine, tyrosine and tryptophan biosynthesis; 2: citrate cycle (TCA cycle); 3: glyoxylate and dicarboxylate metabolism; 4: glycine, serine and threonine metabolism; 5: biosynthesis of unsaturated fatty acids; 6: arginine and proline metabolism; 7: phenylalanine metabolism; 8: alanine, aspartate and glutamate metabolism; 9: glycerophospholipid metabolism; 10: linoleic acid metabolism; 11: arginine biosynthesis; 12: butanoate metabolism; 13: pyruvate metabolism; 14: glycolysis/gluconeogenesis; 15: galactose metabolism; 16: neomycin, kanamycin and gentamicin biosynthesis; 17: arachidonic acid metabolism; 18: lipoic acid metabolism; 19: glutathione metabolism; 20: alpha-Linolenic acid metabolism).

Proline metabolism, Phenylalanine metabolism, Alanine, Aspartate, and Glutamate metabolism, Glycerophospholipid metabolism, Linoleic acid metabolism, Arginine biosynthesis, Butanoate metabolism, and Pyruvate metabolism.

The top four metabolic pathways exerting influence on the occurrence of CHD were systematically chosen for in-depth analysis. These pathways include Phenylalanine, Tyrosine, and Tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, and Glycine, Serine, and Threonine metabolism. Figs. 3–6 provide detailed representations of the structures and metabolic pathways associated with these four pathways exhibiting higher impact values.

Fig. 3 delineates the metabolic pathway structure and KEGG pathway diagram of Phenylalanine, Tyrosine, and Tryptophan biosynthesis. The original *P*-value for the entire pathway was 1.75E-04.

Table 3
The MetPA analysis results of metabolic products in CHD patients.

Pathway name	Total number of compounds	Number of matches	Raw *P*	Impact	FDR	Details
Phenylalanine, tyrosine, and tryptophan biosynthesis	4	3	1.75E-04	1	0.014035	KEEG SMP
Citrate cycle (TCA cycle)	20	5	5.32E-04	0.27299	0.017864	KEEG
Glyoxylate and dicarboxylate metabolism	32	6	7.52E-04	0.13757	0.017864	KEEG
Glycine, serine, and threonine metabolism	33	6	8.93E-04	0.40733	0.017864	KEEG
Biosynthesis of unsaturated fatty acids	36	6	0.001444	0	0.019246	KEEG SMP
Arginine and proline metabolism	36	6	0.001444	0.35581	0.019246	KEEG
Phenylalanine metabolism	8	3	0.002213	0.61904	0.025296	KEEG SMP SMP
Alanine, aspartate, and glutamate metabolism	28	5	0.0027	0.04808	0.026997	KEEG SMP
Glycerophospholipid metabolism	36	5	0.008296	0.22885	0.073743	KEEG SMP
Linoleic acid metabolism	5	2	0.011998	1	0.089607	KEEG SMP
Arginine biosynthesis	14	3	0.012321	0.13705	0.089607	KEEG SMP
Butanoate metabolism	15	3	0.01501	0	0.10007	KEEG SMP
Pyruvate metabolism	23	3	0.047627	0.27088	0.29309	KEEG SMP

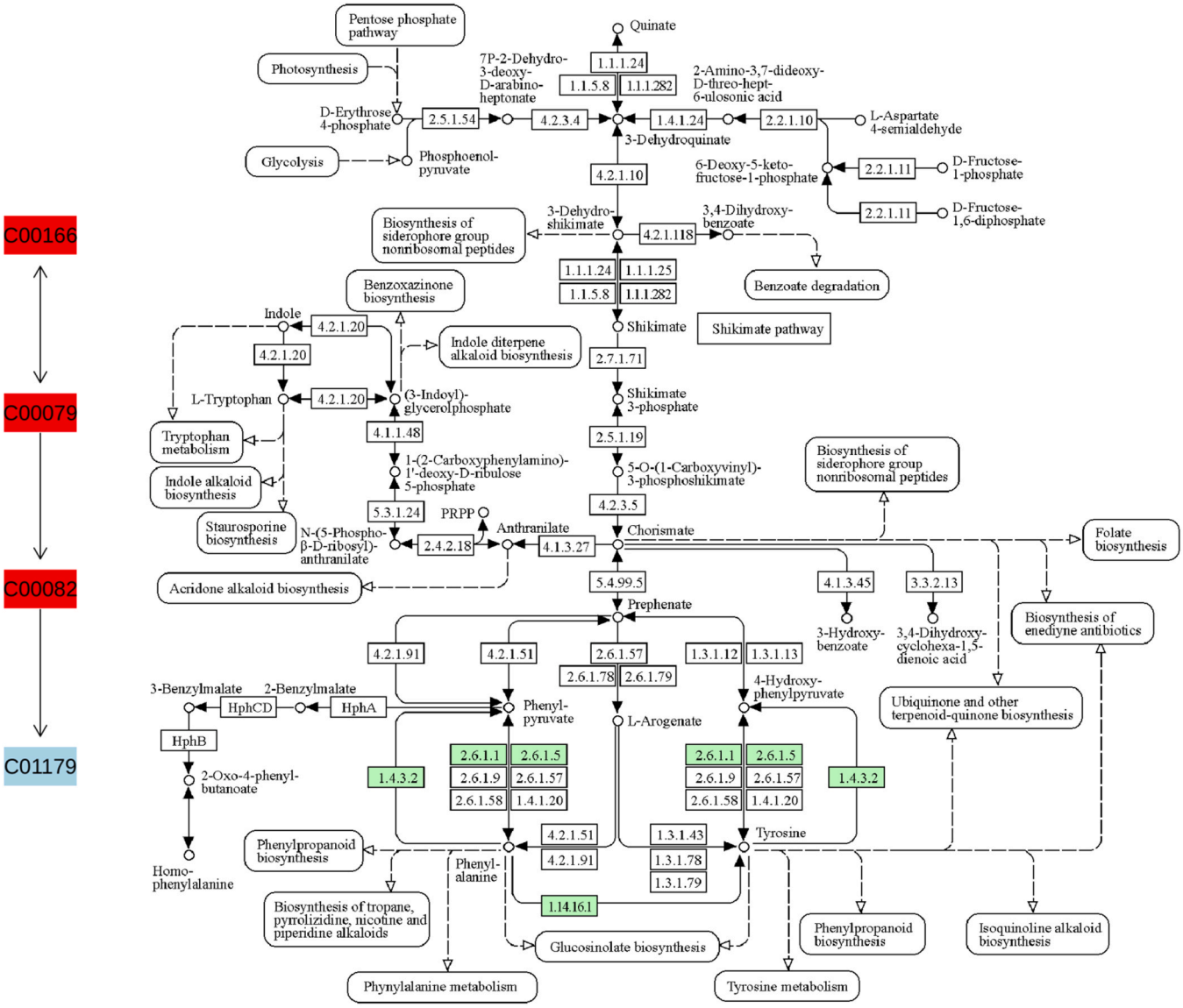

Fig. 3. The structural diagram of Phenylalanine, tyrosine, and tryptophan biosynthesis pathway. (In the figures, the KEGG IDs are presented, and metabolites relevant to this study are highlighted with either a red or green background.).

Notable metabolites within this pathway include phenylpyruvic acid (C00166), Phe (C00079), and tyrosine (C00082). Tyrosine is positioned downstream of phenylpyruvic acid and Phe in the metabolic sequence.

Fig. 4 delineates the metabolic pathway structure and KEGG pathway diagram of the Citrate cycle (TCA cycle). The original *P*-value for the entire pathway was 5.32E-04. Key metabolites participating in this pathway include Pyruvate (C00022), Succinic acid (C00042), citric acid (C00158), Isocitric acid (C00311), and oxoglutaric acid (C00026).

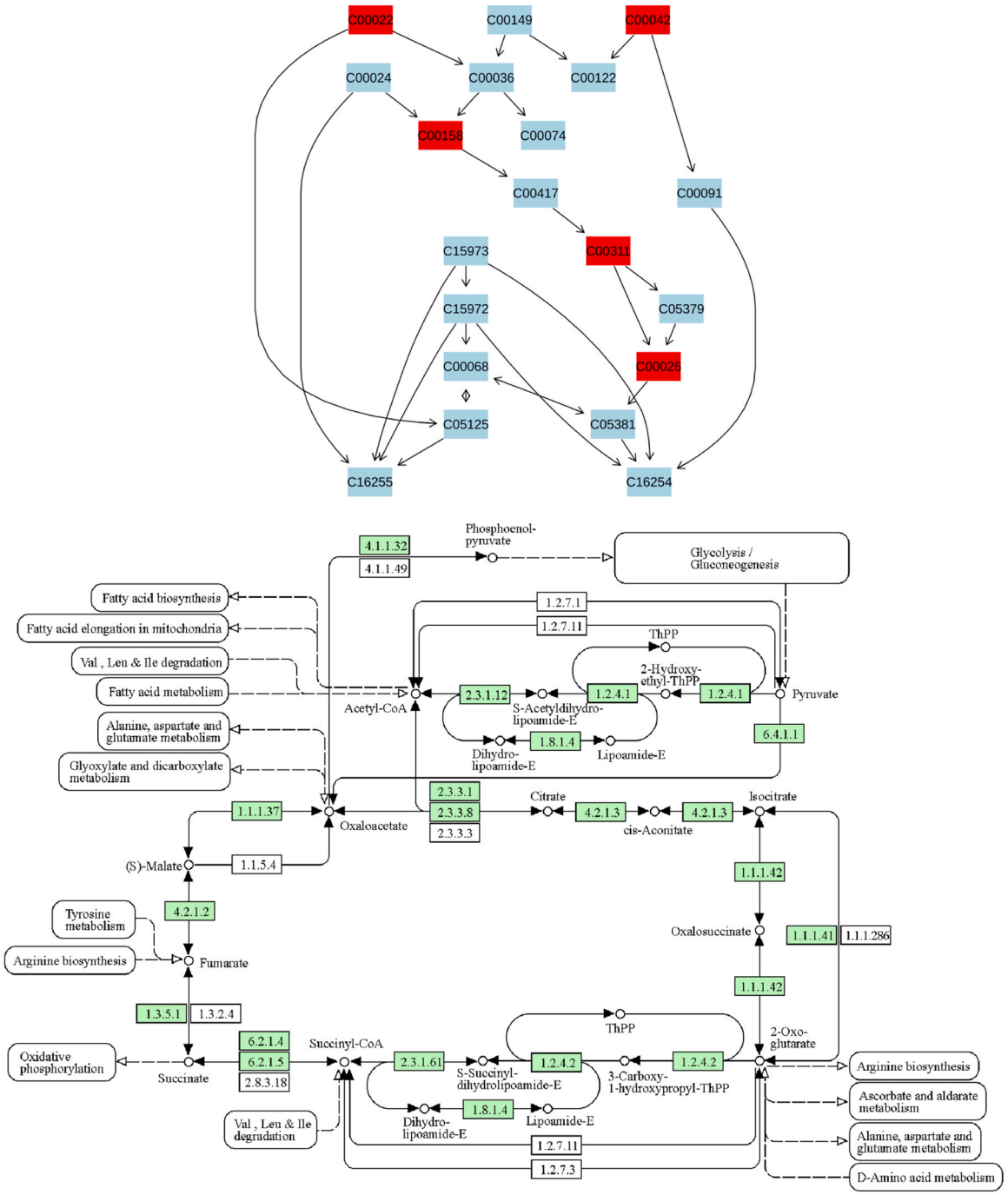

Fig. 4. Structure of Citrate cycle (TCA cycle) metabolic pathway. (In the figures, the KEGG IDs are displayed, and metabolites involved in this study are indicated with a red background.).

Of significance, oxoglutaric acid is situated downstream of Pyruvate, citric acid, and Isocitric acid in the metabolic cascade.

Fig. 5 depicts the metabolic pathway structure and KEGG pathway diagram of glyoxylate and dicarboxylate metabolism. The original *P*-value for the entire glyoxylate and dicarboxylate metabolic pathway was 7.52E-04. Key metabolites engaged in this pathway include acetic acid (C00033), citric acid (C00158), Isocitric acid (C00311), formate (C00058), Pyruvate (C00022), and glycine (C00037).

Fig. 6 illustrates the metabolic pathway structure and KEGG pathway diagram of Glycine, Serine, and Threonine metabolism. The original *P*-value for the entire Glycine, Serine, and Threonine metabolism pathway was 8.93E-04. Metabolites participating in this pathway include Choline (C00114), DL-O-phosphoserine (C01005), dimethylglycine (C01026), Pyruvate (C00022), glycine (C00037), and Glycocyamine (C00581). Notably, Glycocyamine is positioned as a downstream metabolite of glycine in this metabolic pathway.

4. Discussion

Metabolomics, as an emerging omics analysis technology in the post-genomic era, enables the analysis of disease pathological processes or drug metabolic pathways using high-efficiency and high-sensitivity detection instruments. With the recent advancements in bioinformatics technology, the analysis of changes in disease metabolites and their interactions holds great significance for understanding the pathogenesis of diseases. Building upon the findings of previous studies, the present

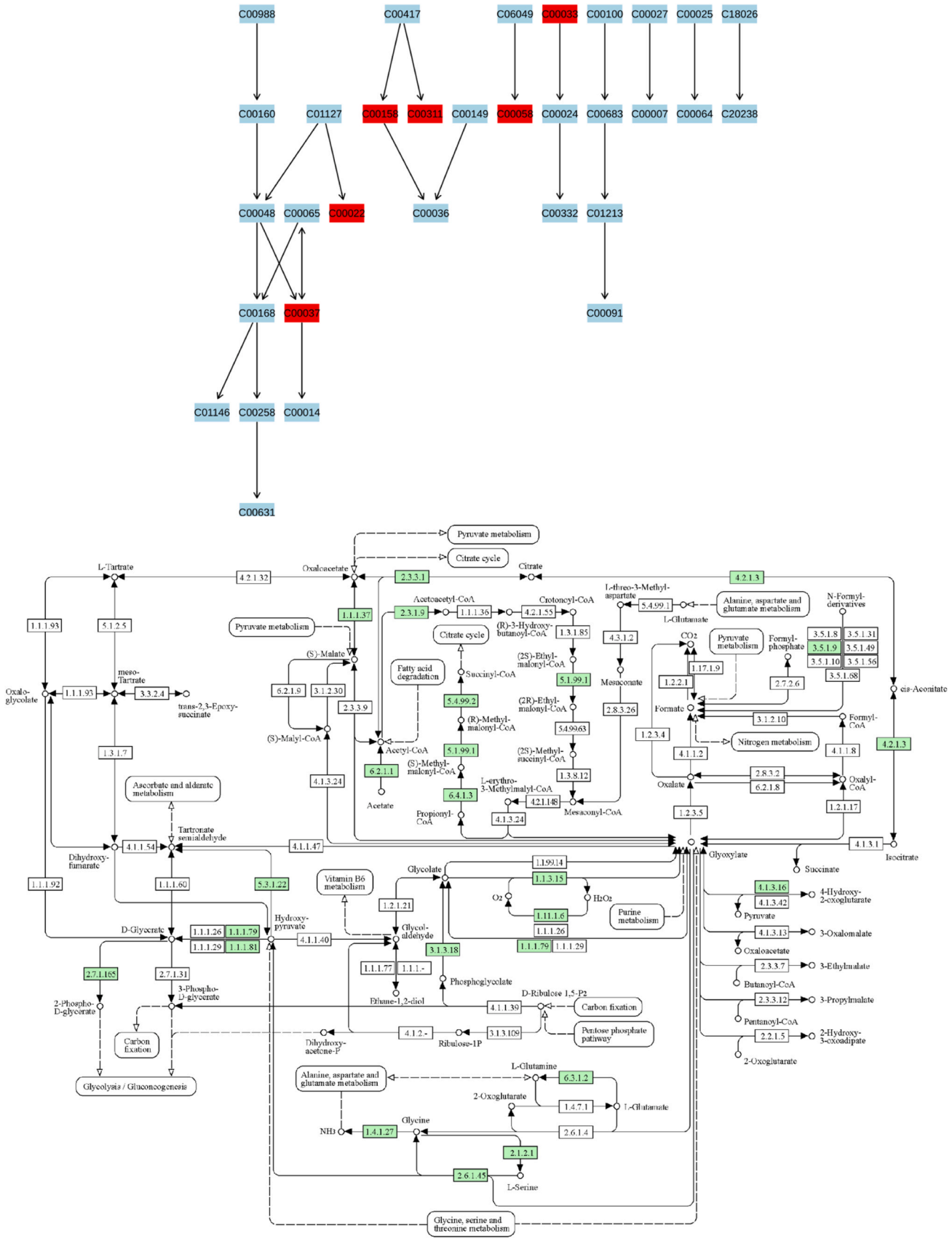

Fig. 5. Structure of glyoxylate and dicarboxylate metabolic pathway. (KEGG ID was shown in the figure, compounded with red or green background are metabolites participating in this work.).

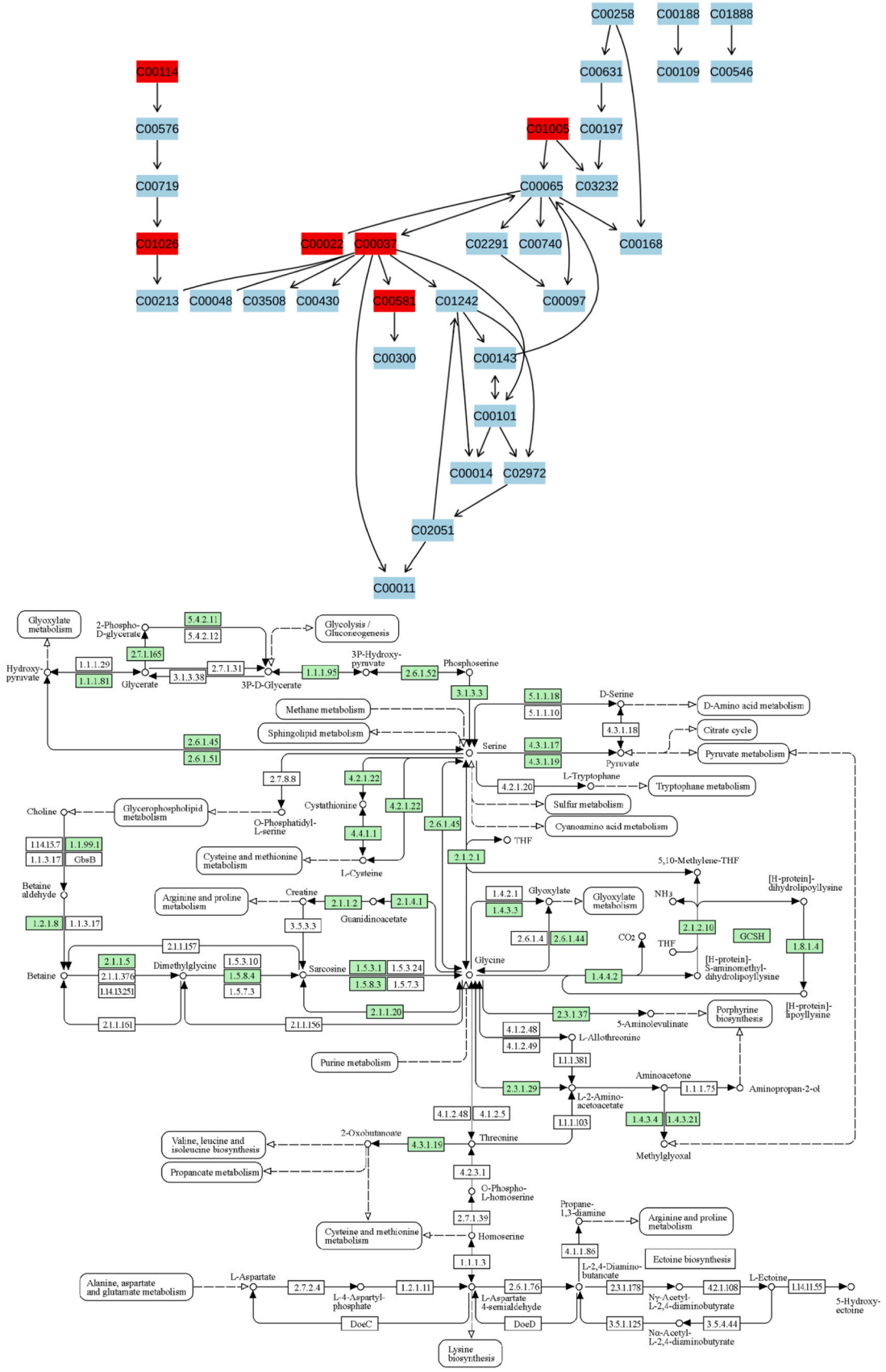

Fig. 6. Structure of Glycine, serine, and threonine metabolism pathway. (In the figures, the KEGG IDs are presented, and metabolites involved in this study are highlighted with either a red or green background.).

research utilized bioinformatics methods to analyze alterations in the metabolome of CHD patients and mapped out associated metabolic pathways. A total of 87 metabolites are involved in 45 metabolic pathways. The Metabolomics Pathway Analysis (metPA) overview diagram provides a visual representation of the significance of metabolic product pathways and their interrelationships with other pathways. Through pathway topology analysis calculating their impact values, 13 main metabolic pathways were selected, namely Phenylalanine, tyrosine and tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, Glycine, serine and threonine metabolism, Biosynthesis of unsaturated fatty acids, Arginine and proline metabolism, Phenylalanine metabolism, Alanine, aspartate and glutamate metabolism, Glycerophospholipid metabolism, Linoleic acid metabolism, Arginine biosynthesis, Butanoate metabolism, Pyruvate metabolism. The topological network diagram of metabolites pathway was established, which provided certain theoretical basis for elucidating CHD mechanism and interaction among metabolites. The top 4 metabolic pathways selected for the analysis of their impact on CHD occurrence are Phenylalanine, tyrosine and tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, and Glycine, serine, and threonine metabolism.

The KEGG metabolic pathway diagram serves as a visual tool to illustrate the relationships and composition of various metabolic pathways within an organism. The KEGG metabolic pathway diagram can depict the molecular constituents and interaction relationships within metabolic pathways. By observing and analyzing these diagrams, researchers can swiftly comprehend the overall structure and underlying mechanisms of metabolic pathways. This aids in elucidating the biological functions and regulatory mechanisms of these pathways. Utilizing KEGG metabolic pathway diagrams, researchers can identify associated metabolic products and pathways, providing clues for the discovery of new metabolites, pathways, and related biological processes. This is pivotal for uncovering novel biosynthetic pathways, identifying new drug targets, and understanding the mechanisms underlying metabolism-related diseases. KEGG metabolic pathway diagrams facilitate the analysis of interactions and regulatory relationships among molecules in the metabolic network. By examining reactions and connections in the metabolic pathway diagrams, the complexity and dynamics of the metabolic network can be revealed, contributing to a comprehensive understanding of the overall regulatory mechanisms of metabolism. In this study, KEGG metabolic pathway diagrams were employed to represent the first four pathways associated with CHD.

Phenylalanine, tyrosine, and tryptophan are three essential aromatic amino acids in the human body. These amino acids function not only as fundamental components of proteins but also actively participate in diverse biological metabolic pathways and signal transduction cascades in organisms. Phenylalanine and tyrosine are precursors for nitric oxide synthesis, and nitric oxide plays a role in vasodilation within blood vessel walls, helping regulate vascular tone. In CHD, nitric oxide synthesis may be influenced [26], and amino acid supply plays a regulatory role in nitric oxide production [27]. Amino acid metabolism is intricately linked to the body's energy metabolism and fibrinolysis [28]. Disturbances in metabolic processes associated with CHD may result in abnormal changes in vascular walls, including the development of atherosclerosis and the formation of atheromatous plaques [29]. CHD is frequently accompanied by chronic inflammation, and aromatic amino acids can actively participate in the inflammatory process by modulating cell signaling pathways involved in inflammation, thereby influencing the development of atherosclerosis [30]. Amino acid metabolism is associated with the antioxidant system, influencing the balance of intracellular oxidative stress. In patients with CHD, oxidative stress may exacerbate cardiovascular lesions [31]. Aromatic amino acids can impact the function of immune cells, including T lymphocytes and macrophages, and the regulation of the immune system may play a role in the development of CHD [32]. The results indicated that three metabolites, phenylpyruvic acid (C00166), Phe (C00079), and tyrosine (C00082), are involved in the Phenylalanine, tyrosine, and tryptophan biosynthesis pathway. Phenylpyruvic Acid (C00166) is an intermediate product in phenylalanine metabolism, playing a role in carrying and transferring carbon skeletons during the phenylalanine metabolism process. Phenylalanine (C00079) is synthesized through the aromatic amino acid pathway, serving not only as a component of protein synthesis but also as a precursor for many bioactive molecules such as adrenaline and thyroxine. Additionally, phenylalanine is the starting material for some biochemical pathways, including the synthesis of tyrosine. Tyrosine (C00082) is derived from phenylalanine and serves as a crucial intermediate in the aromatic amino acid metabolic pathway. It is a synthesis precursor for various important biomolecules such as adrenaline, noradrenaline, and thyroid hormones. Additionally, tyrosine is involved in the nitric oxide (NO) synthesis pathway, playing a role in regulating vascular tension. Current research results indicate that tyrosine can stimulate the production of catecholamines in the brain, thereby physiologically lowering blood pressure [33]. Nitric oxide (NO) has a vasodilatory effect in the endothelium, providing protective effects on the cardiovascular system. However, when the production of NO increases, it may coincide with an increase in superoxide production, leading to lipid peroxidation. This peroxidation process may negatively impact cellular components and accelerate the development of atherosclerosis [34].

The citric acid cycle, also known as the tricarboxylic acid (TCA) cycle, is a foundational metabolic pathway ubiquitous in aerobic organisms. This pathway involves a series of biochemical reactions within the cell that culminate in the breakdown of organic substances into carbon dioxide and energy. Widely recognized as a central and ultimate metabolic pathway for the decomposition of sugars, fats, and proteins, the TCA cycle tightly interconnects the metabolism of these biomolecules. This cycle holds paramount significance as a critical component of cellular respiration, transpiring in the mitochondrial matrix within the cytoplasm. As the fundamental process governing cellular biochemical energy supply, the TCA cycle undergoes alterations in various cardiovascular diseases. In the context of CHD, the function of the citric acid cycle may be influenced, thereby directly or indirectly contributing to the development of the disease [35,36]. The citric acid cycle is one of the primary pathways for ATP generation within cells. In patients with CHD, myocardial cells may be influenced by factors such as ischemia and hypoxia, leading to an imbalance in energy demand and supply. The citric acid cycle, through the oxidation of glucose, fats, and amino acids, provides the required ATP for the myocardium. However, when the cycle is disrupted, it may impact the heart's function. The reduced coenzymes generated by the citric acid cycle, such as NADH and $FADH_2$, participate in intracellular redox reactions through the oxidative phosphorylation process within mitochondria. These coenzymes to some extent contribute to combating oxidative stress and providing protection, while CHD is often associated with an increase in oxidative stress. The citric acid cycle is closely associated with amino acid metabolism, as certain amino acids can enter metabolic pathways through the citric acid cycle, generating energy. In the context of CHD, potential abnormalities in amino acid metabolism may give rise to alterations in the citric acid cycle. Oxalic acid, an intermediate product within the citric acid cycle, could be influenced by disruptions in the cycle, impacting its generation. Notably, oxalic acid has been recognized for its potential protective effect on cardiovascular health, attributed to its role as an antioxidant. Furthermore, the citric acid cycle plays a crucial role in the elimination of metabolic waste, including carbon dioxide, from the body. In CHD, disturbances in the elimination of metabolic waste may have repercussions on acid-base balance and the stability of the intracellular environment. Additionally, certain traditional Chinese medicines have been suggested in studies to possess anti-myocardial ischemia effects, with mechanisms potentially involving the regulation of the citric acid cycle pathway [37,38]. This indicates that the citric acid cycle plays a crucial role in both cardiac health and disease processes, offering valuable clues for exploring new approaches to the treatment of heart

diseases.

The results indicated the involvement of five metabolites—Pyruvate (C00022), Succinic acid (C00042), citric acid (C00158), Isocitric acid (C00311), and oxoglutaric acid (C00026)—in the TCA cycle metabolic pathway. Pyruvate (C00022) is one of the final products of glycolysis. In CHD, the regulation of pyruvate metabolism is governed by multiple biological processes, encompassing energy supply, oxidative stress, and inflammation. Serving as a pivotal component in mitochondrial oxidative phosphorylation, pyruvate actively engages in the citric acid cycle and the respiratory chain to generate ATP, thereby supplying essential energy for cardiac cells. In instances of CHD, myocardial cells may encounter insufficient oxygen supply, leading to alterations in pyruvate metabolism and subsequently impacting energy production. The metabolism of pyruvate is intricately linked with the metabolic pathways of glucose, fatty acids, and amino acids. In the context of CHD, metabolic reprogramming within myocardial cells induces changes in the production and utilization of pyruvate [39]. CHD often accompanies an increase in oxidative stress, leading to the disturbance of intracellular redox balance. Pyruvate (C00022) can generate cofactors through the citric acid cycle, participating in intracellular redox reactions and exhibiting certain antioxidant effects. CHD is frequently correlated with chronic inflammation, and the regulation of pyruvate metabolism may contribute to modulating the inflammatory processes associated with this condition. Recent studies have suggested that interventions targeting pyruvate metabolism could hold therapeutic potential for cardiovascular diseases, indicating a promising avenue for future research and therapeutic development [40]. Succinic acid (C00042) is involved in energy metabolism, antioxidant effects, and the regulation of inflammatory processes. Succinic acid is an intermediate product in the citric acid cycle, and through the metabolism of the citric acid cycle, it can generate cofactors NADH and $FADH_2$, providing the necessary energy for cardiac cells. Owing to its antioxidant properties, succinic acid functions as a reducing agent within cells, mitigating oxidative stress and potentially ameliorating oxidative damage in the context of CHD. In instances of CHD, where myocardial cells may encounter inadequate oxygen supply, the regulation of succinic acid metabolism becomes pivotal in sustaining intracellular energy balance. Succinic acid serves as a source of energy, thereby supporting the normal functioning of the heart. Moreover, CHD is frequently accompanied by inflammation, and the metabolism of succinic acid may be intricately linked to the inflammatory process. Through the regulation of specific signaling pathways, the metabolism of succinic acid could influence inflammatory reactions, offering insights into potential therapeutic avenues for managing inflammation in CHD [41]. Modulating the metabolic pathways of succinic acid can have a protective effect on CHD [42]. Citric acid (C00158) participates in energy metabolism, antioxidant effects, and the regulation of inflammation processes. Citric acid serves as the primary substrate in the initiation of the citric acid cycle, and through its metabolic processes, the cycle generates cofactors NADH and $FADH_2$, thereby supplying energy for cardiac function and manifesting antioxidant effects. The metabolism of citric acid is implicated in the potential regulation of the inflammatory process, showcasing a complex interplay between the citric acid cycle, cellular metabolism, and inflammatory responses. Being an acidic substance, citric acid may play a role in maintaining acid-base balance within cells. In cases of CHD, where the metabolism and function of myocardial cells may be compromised, the regulatory role of citric acid assumes significance, potentially influencing the stability of the intracellular environment. Isocitric acid (C00311) is an intermediate product in the citric acid cycle, and its role in CHD may be manifested through involvement in energy metabolism, antioxidant effects, and the regulation of inflammation pathways [43]. α-Ketoglutaric acid, generated from isocitric acid, produces cofactors NADH and $FADH_2$, providing energy for the heart and exerting antioxidant effects, offering protection against oxidative stress. In cases of CHD, myocardial cells may experience the impact of hypoxia and energy deficiency. α-Ketoglutaric acid, by virtue of its involvement in energy metabolism, plays a significant role in maintaining normal function and energy balance within myocardial cells. Oxoglutaric acid (C00026), a pivotal intermediate product in the citric acid cycle, undergoes cyclical metabolism, leading to the production of cofactors NADH and $FADH_2$. This process not only supplies energy but also imparts antioxidant properties to the heart. Given the challenges of oxygen and energy deficiency encountered by myocardial cells in CHD, α-Ketoglutaric acid, through its active participation in energy metabolism, contributes to sustaining normal function and energy balance in these cells.

Glyoxylate and dicarboxylate metabolism is a biochemical metabolic pathway involving the synthesis and degradation of a series of organic acids, particularly crucial in microorganisms. CHD is a complex cardiovascular disorder involving various biochemical processes and metabolic pathways. Generally, cardiovascular health is associated with factors such as cellular energy metabolism, antioxidant capacity, and inflammation. While current research findings do not explicitly establish a direct correlation between the Glyoxylate and dicarboxylate metabolism pathway and the onset of CHD, this pathway demonstrates significant associations with lipid metabolism, glucose metabolism, amino acid metabolism, and various other processes [44]. In the progression of CHD, critical biological processes, including cellular energy metabolism, oxidative stress, and inflammation, play pivotal roles. Future investigations may elucidate further connections between additional metabolic pathways and CHD. Recent research results indicate that the treatment of CHD induces alterations in the Glyoxylate and dicarboxylate metabolism pathway, suggesting a potential role for this pathway in the pharmacological treatment of CHD [45]. The results showed that metabolites such as Choline (C00114), DL-O-phosphoserine (C01005), dimethylglycine (C01026), Pyruvate (C00022), glycine (C00037), and Glycocyamine (C00581) are involved in the Glyoxylate and dicarboxylate metabolism pathway. Choline (C00114) is a crucial nutrient involved in maintaining cell structure, neural transmission, and lipid metabolism. Choline serves as a precursor to phospholipids, especially phosphatidylcholine, a vital component of cell membranes. A well-structured membrane is essential for maintaining cell integrity and function. Disruption in lipid metabolism may be associated with the development of atherosclerosis, serving as a crucial factor in CHD [46]. By regulating lipid metabolism, preventive measures against CHD can be implemented [47]. Choline, through its metabolic processes, provides methyl donors, participating in the body's one-carbon metabolism pathway, which is associated with various biological processes such as DNA methylation and protein synthesis. Studies suggested that disruptions in one-carbon metabolism may be linked to an increased risk of CHD [48]. Choline exhibits anti-inflammatory properties and can suppress inflammatory reactions by modulating cell membrane structure and signaling pathways. Chronic inflammation is closely related to the development of atherosclerosis and CHD. One of the metabolites of choline metabolism is Trimethylamine N-oxide (TMAO), and elevated levels of TMAO are associated with an increased risk of CHD [49]. TMAO may impact CHD by influencing cholesterol metabolism and the development of atherosclerosis. Current research indicated that choline participates in the occurrence and development of CHD through lipid metabolism, one-carbon metabolism, anti-inflammatory effects, and other aspects [50]. DL-O-phosphoserine (C01005) is an amino acid and its phosphorylated product, typically involved in post-translational modification of proteins and some cell signaling pathways. CHD is a complex cardiovascular disorder involving various biological processes, including but not limited to disruptions in lipid metabolism, inflammation, and endothelial dysfunction. DL-phosphoserine, as a metabolic product, is postulated to have implications in protein synthesis, signaling pathways, and various metabolic processes. However, a more comprehensive and detailed investigation is warranted to elucidate its specific relationship with CHD. Dimethylglycine (C01026), a derivative of glycine, is abundantly present in various foods and can also be endogenously produced through metabolic processes within the body. Given its composition with methyl groups, dimethylglycine may be

intricately associated with the one-carbon metabolism pathway. One-carbon metabolism is intimately linked to processes such as homocysteine regulation, DNA methylation, and the generation of methyl donors, all of which play critical roles in cardiovascular health. Research suggested that Dimethylglycine possesses certain antioxidant properties, capable of alleviating oxidative stress-induced damage to the cardiovascular system. Oxidative stress is closely associated with the occurrence and development of CHD. Glycine (C00037) is an antioxidant that helps mitigate oxidative stress-induced damage to cells and tissues. Oxidative stress is implicated in the development of atherosclerosis and CHD, and the role of antioxidants may contribute to protecting the cardiovascular system. Glycine can also regulate the concentration of nitric oxide (NO), thereby improving arterial stiffness and modulating central arterial blood pressure. Some studies indicate that glycine has inhibitory effects on inflammatory responses. Chronic inflammation stands as a pivotal factor in the pathogenesis of CHD, and the potential anti-inflammatory effects of glycine may contribute to impeding the progression of CHD. Glycine, as a constituent of the one-carbon metabolism pathway, is intricately associated with processes such as DNA methylation. Disruptions in one-carbon metabolism have been implicated in the development of CHD, and glycine is postulated to play a regulatory role in this intricate process. Glycocyamine (C00581), a compound convertible to creatine within the body, is noteworthy in the context of muscle energy metabolism. Creatine, derived from glycocyamine, is recognized for its association with muscle energy metabolism and may play a significant role in related physiological processes. Glycocyamine can undergo conversion to creatine in the body, and creatine is involved in the cellular process of phosphorylation, storing and releasing energy. Creatine phosphorylation is a mechanism for storing and transferring energy during high-intensity exercise, and it is related to myocardial energy metabolism and cardiac function. Glycocyamine and its conversion product, creatine, are widely used for improving athletic performance, particularly in high-intensity and short-duration exercises [51]. Physical activity and exercise are beneficial for cardiovascular health, and therefore, Glycocyamine may have a positive impact on the cardiovascular system by enhancing exercise capacity.

Glycine, serine, and threonine metabolism are crucial metabolic pathways in cells, involving the synthesis and breakdown of these three amino acids. This metabolic pathway plays a key role in maintaining amino acid balance, protein synthesis, one-carbon metabolism, and other essential cellular processes. The metabolism of glycine, serine, and threonine is linked to the one-carbon metabolism pathway [52,53]. One-carbon metabolism constitutes a fundamental cellular process, contributing to various biological phenomena, including DNA methylation and protein synthesis. Dysregulation of one-carbon metabolism has been linked to cardiovascular diseases, notably coronary artery disease. The Glycine, Serine, and Threonine metabolism pathway intricately involves nitrogen metabolism and protein synthesis. Perturbations in nitrogen metabolism and protein synthesis may exert an impact on cardiovascular health, particularly in the context of risk factors associated with coronary artery disease, such as atherosclerosis. The Glycine, Serine, and Threonine metabolism pathway also play a role in energy metabolism, particularly concerning the metabolism of serine, which is associated with energy regulation. Given the high energy demands of the heart, abnormalities in energy metabolism may potentially contribute to the pathogenesis of coronary artery disease. Furthermore, the metabolism of specific amino acids is intertwined with immune regulation and inflammatory responses. Chronic inflammation has been intricately linked to the development of coronary artery disease. Understanding the complex interplay of these metabolic pathways provides insights into the multifaceted mechanisms underlying cardiovascular health and disease. The results indicated that Choline (C00114), DL-O-phosphoserine (C01005), dimethylglycine (C01026), Pyruvate (C00022), glycine (C00037), and Glycocyamine (C00581) participate in the Glycine, serine, and threonine metabolism pathway. These six metabolites have direct or indirect roles in the occurrence and development of coronary artery disease.

5. Conclusion

Utilizing bioinformatics analysis, this study delved into the interactions and biological pathways of metabolomic products in patients with CHD. Thirteen pertinent literatures from current metabolomic studies were included, and 87 potential differential metabolites were identified. KEGG analysis unveiled that these differential metabolites primarily participated in 13 metabolic pathways. The occurrence of CHD is mainly associated with the metabolic pathways of Phenylalanine, tyrosine and tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, and Glycine, serine, and threonine metabolism. These pathways are primarily involved in the regulation of processes such as inflammation, oxidative stress, one-carbon metabolism, energy metabolism, lipid metabolism, immune regulation, and nitric oxide expression. However, this study acknowledges some limitations. It only provides an initial exploration of the correlation and pathways among differential metabolites in CHD patients. The study did not analyze the differences in metabolites among patients with varying severity of CHD. Future work will further analyze the distinctions in metabolites among CHD patients with different severity levels to offer reference points for the diagnosis and treatment of CHD. It is evident that Phenylalanine, tyrosine and tryptophan biosynthesis, Citrate cycle (TCA cycle), Glyoxylate and dicarboxylate metabolism, and Glycine, serine, and threonine metabolism pathways all play a role in the occurrence of CHD. The development of CHD is associated with processes and the regulation of metabolites related to inflammation, oxidative stress, one-carbon metabolism, energy metabolism, lipid metabolism, immune regulation, and nitric oxide expression. This provides theoretical references for understanding the pathogenesis and formulating treatment strategies for CHD.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

CC, SL,LN,HH,YL, and JY designed the research, completed all analysis in the research, drafted the manuscript, participated in the manuscript's data collection and literal modification and wrote the original draft. All authors read and approved the final manuscript.

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CRediT authorship contribution statement

Shengquan Liu: Writing – original draft, Visualization, Investigation, Conceptualization. **Liangui Nie:** Writing – original draft, Validation, Investigation, Conceptualization. **Chun Chu:** Writing – original draft, Methodology, Investigation, Conceptualization. **Jun Yang:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Hongming Hu:** Writing – original draft, Methodology, Investigation, Conceptualization. **Yi Liu:** Validation, Investigation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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