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Research Article

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Discovery of potential biomarkers for human atherosclerotic abdominal aortic aneurysm through untargeted metabolomics and transcriptomics

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Abstract: Abdominal aortic aneurysm (AAA) and atherosclerosis (AS) have considerable similarities in clinical risk factors and molecular pathogenesis. The aim of our study was to investigate the differences between AAA and AS from the perspective of metabolomics, and to explore the potential mechanisms of differential metabolites via integration analysis with transcriptomics. Plasma samples from 32 AAA and 32 AS patients were applied to characterize the metabolite profiles using untargeted liquid chromatography-mass spectrometry (LC-MS). A total of 18 remarkably different metabolites were identified, and a combination of seven metabolites could potentially serve as a biomarker to distinguish AAA and AS, with an area under the curve (AUC) of 0.93. Subsequently, we analyzed both the metabolomics and transcriptomics data and found that seven metabolites, especially 2'-deoxy-D-ribose (2dDR), were significantly correlated with differentially expressed genes. In conclusion, our study presents a comprehensive landscape of plasma metabolites in AAA and AS patients, and provides a research direction for pathogenetic mechanisms in atherosclerotic AAA.

Key words: Abdominal aortic aneurysm (AAA); Atherosclerosis (AS); Untargeted metabolomics; Transcriptomics

1 Introduction

Abdominal aortic aneurysm (AAA) is defined as a localized, permanent, and irreversible dilation of the abdominal aorta beyond 50% of the normal vessel diameter (Subcommittee on Reporting Standards for Arterial Aneurysms et al., 1991). AAA is a complex disease mainly occurring in the aging population and associated with a mortality rate of 85%–90% after rupture (Alcorn et al., 1996; Kent, 2014). Atherosclerosis (AS) is well known to contribute to the occurrence of AAA, which shares common risk factors such as smoking, increasing age, family history, and male sex (Ito et al., 2008). The main pathological features of AAA include chronic inflammation, vascular smooth muscle cell apoptosis, extracellular matrix degradation, and thrombosis (Wassef et al., 2001; Pearce and Shively, 2006), which are also involved in vulnerable atherosclerotic plaque formation, illustrating that AS may be an important driver for AAA (Cornuz et al., 2004; Palazzuoli et al., 2008). However, genome-wide sequencing has revealed that although AAA and AS have similar genetic pathways in lipid metabolism, smooth muscle cell function, and inflammation regulation, there are a large number of independent pathogenic gene loci (Bradley et al., 2013; Harrison et al., 2013; Jones et al., 2013; Leeper et al., 2013). Currently, there is no general agreement about the

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relationship between AAA and AS, and thus it is necessary to explore the differences between these two diseases from other perspectives.

Recent studies have indicated that dietary pattern is associated with AAA risk, and diet affects the body through metabolites (Haring et al., 2018; Kaluza et al., 2019). Metabolomics is the newest member of the omics trilogy and has become a powerful tool for functional genomics research in the post-genomics era (Zampieri et al., 2017). Metabolomics is commonly divided into two types, including targeted and untargeted analyses. Targeted metabolomics is mostly used to identify and quantify a limited number of known metabolites. In contrast, untargeted metabolomics can explore far more species, whether known or unknown. The major advantage of untargeted metabolomics is the potential identification of new metabolites and pathways linked to pathophysiological processes and disease susceptibility (Johnson et al., 2016; Schrimpe-Rutledge et al., 2016).

During the past decade, extensive research has applied untargeted metabolomics to cardiovascular diseases, including AS (Ussher et al., 2016). Several metabolites have been shown to affect the progression of AS. For example, trimethylamine *N*-oxide (TMAO) is a small organic compound whose concentration in blood increases after ingesting dietary L-carnitine and phosphatidylcholine (Ufnal et al., 2015). An untargeted metabolomic study identified that TMAO was mechanistically linked with cardiovascular disease risk in humans (Li et al., 2018). Compared with AS, only a few metabolomic studies have been carried out on AAA (Qureshi et al., 2017). A group of linoleic acid-containing triacylglycerols and diacylglycerols were shown to be increased in AAA patients (n=161) compared to patients with peripheral artery disease (n=168) using targeted metabolomics (Moxon et al., 2014). However, the research has been restricted to limited comparisons of lipids. In addition, it is also important to realize that metabolic fluxes are regulated by environmental stresses in addition to gene expression; in other words, metabolomics is a level downstream from transcriptomics (Macel et al., 2010). Gene expression profiling is conducive to understanding the molecular mechanisms of complex disorders, including heart disease and metabolic disorders (Kim et al., 2010). By comparing the transcriptomic profiles of AAA patients and aortic occlusive disease (AOD) patients, Biros et al. (2015) found that immune-related pathways and several important genes (such as cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*), natural killer cell triggering receptor (*NKTR*), and cluster of differentiation 8A (*CD8A*)) were involved in AAA pathogenesis. A recent study further explored the gene expression patterns for tunica and media. Lipid-related processes in the adventitia were found to be correlated with AAA growth rate (Lindquist Liljeqvist et al., 2020). Integration of transcriptomics and metabolomics can screen a large number of metabolites so as to identify the most important metabolites between AAA and AS.

The aim of the present study is to throw light on the differential metabolic profiling between AAA and AS using untargeted liquid chromatography-mass spectrometry (LC-MS), and then to identify potential mediators for distinguishing AAA and AS patients. Moreover, we combined analysis of differential metabolites with gene expression profiling from the database to explore the crucial metabolites and genes in the progression of atherosclerotic AAA.

2 Materials and methods

2.1 Patient recruitment and clinical data collection

All patients were recruited from the Department of Vascular Surgery, Peking Union Medical College Hospital (Beijing, China). Patients with AAA and AS diagnosed by ultrasound or computed tomography (CT) were included. Non-atherosclerotic aneurysms (infectious or inflammatory) were excluded. In order to ensure the consistency of the AS group, only patients with carotid atherosclerotic stenosis were selected. The degree of stenosis was determined according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) standard (NASCET collaborators, 1991), and all AS patients were at or above 70% of the standard. Patients with known history of disease with an autoimmune component (such as inflammatory bowel disease), history of malignancy, or any digestive tract disease and surgery were excluded.

Demographic and clinical data were collected from patients. The enumeration data were described in terms of frequency and percentage, and the measurement data were generally represented by mean± standard deviation (SD). Normally distributed variables between two groups were analyzed by Student's *t*-test. The Mann-Whitney *U* test was applied for data

of this type that were not normally distributed. Categorical variables were compared by the χ^2 test. All these analyses were carried out with GraphPad Prism 7.04 software (GraphPad Inc., CA, USA). Differences were considered to be statistically significant when P was <0.05.

2.2 Sample collection

Peripheral blood samples were collected in the morning after an overnight fast (≥8 h). Plasma samples were obtained by centrifugation at 3000 r/min (1692 relative centrifugal force) for 10 min at room temperature (ThermoFisher, Sorvall ST 16R, Germany), and then rapidly frozen and stored at −80 °C until analyzed.

2.3 LC-MS analysis

Plasma samples from the included patients were prepared for ultra-high-performance liquid tandem chromatography-quadrupole time of flight mass spectrometry (UHPLC-QTOFMS) analysis by application of validated protocols (Dunn et al., 2011). The LC-MS data were analyzed by SMICA (Version 15.0.2; Sartorius Stedim Data Analytics AB, Umea, Sweden) to conduct multivariate statistical analysis (MVA). Peak devolution, alignment, and integration were applied and the minimum fraction (Minfrac) and cut-off were set as 0.5 and 0.3, respectively, as the stringency. We applied an in-house tandem mass spectrometry (MS2) database which was constructed based on the Human Metabolome Database (HMDB, https://hmdb.ca), MassBank of North America (MoNA, https://mona.fiehnlab.ucdavis.edu), and METLIN (https://metlin.scripps.edu) databases for metabolite identification. Differential metabolites were obtained by comparing AAA and AS patients using Student's t-test. We also conducted Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis for these differential metabolites. Receiver operator characteristic curve (ROC) analysis was performed and linear Support Vector Machine (SVM) analysis was conducted using the Biomarker analysis section of MetaboAnalyst (Version 3.0; https://www.metaboanalyst.ca). The area under curve (AUC) was calculated to show the potential diagnostic value for differential metabolites.

2.4 Transcriptomic profiling analysis

To enable more in-depth understanding of the pathogenetic differences between AAA and AS from a multi-omics perspective, we also acquired transcriptomic profiling data for AAA and AS samples from dataset GSE57691 in the Gene Expression Omnibus (GEO) database. This dataset contains an independent cohort of patients and includes tissue samples instead of plasma samples, as we used. We downloaded the series matrix file and annotated the probe IDs with gene symbols using the GPL10558 platform data table to obtain a gene expression matrix. The "limma" R package (Version 3.48.1) was used to conduct differentially expressed gene (DEG) screening and screened DEGs were subjected to gene ontology (GO) and KEGG pathway analyses using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) V6.8 (https://david.ncifcrf.gov) to obtain the biological functions of these DEGs.

2.5 Joint analysis of metabolomics and transcriptomics

To integrate the multi-omics data, we calculated the Spearman's correlation indices for differential omics data and visualized it with a heatmap (Shannon et al., 2003).

3 Results

3.1 Clinical characteristics of AAA and AS patients

To compare the differences in plasma metabolites between AAA and AS, 32 AAA and 32 AS patients were included in this study. The clinical characteristics of these participants are shown in Table 1 (Ji et al., 2021). Patients with AAA were slightly older (P=0.019), but there were no differences in gender, body mass index (BMI), or underlying diseases such as hypertension and diabetes, which would affect metabolism. Plasma concentrations of C-reactive protein (CRP) and homocysteine (Hcy) did not differ statistically. In addition, we examined the blood lipid profile and found that the total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) of the AAA group were higher than those of the AS group, which may be due to the use of lipid-lowering drugs. For AAA patients, the maximum aneurysm diameter was (5.3±1.4) cm, and the involved length was (7.8±3.3) cm.

Table 1 Clinical characteristics of the two groups

Characteristics	AAA (n=32)	AS (n=32)	P-value
Age (year)	68.6±5.7	64.5±6.7	0.019*
Male	26 (81.3%)	28 (87.5%)	0.491
BMI (kg/m^2)	24.2±3.5	24.7±2.7	0.573
Hypertension	18 (56.3%)	12 (37.5%)	0.133
Diabetes	7 (21.9%)	6 (18.8%)	0.756
CRP (mg/dL)	4.2±10.2	1.6±2.4	0.155
Hcy (μmol/L)	19.0±7.3	16.6±6.3	0.182
TC (mmol/L)	4.5±1.3	3.2±0.7	<0.001*
TG (mmol/L)	1.5±0.9	1.2±0.6	0.469
HDL-C (mmol/L)	1.0±0.2	1.0±0.2	0.550
LDL-C (mmol/L)	2.9±1.1	1.8±0.6	<0.001*
Diameter (cm)	5.3±1.4	N/A	N/A
Length (cm)	7.8±3.3	N/A	N/A

Data are expressed as mean±standard deviation (SD) or number (percentage). * represents the difference was statistically significant. AAA: abdominal aortic aneurysm; AS: atherosclerosis; BMI: body mass index; CRP: C-reactive protein; Hcy: homocysteine; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; N/A: not available.

3.2 Metabolic profiling and functional analysis

In all the samples from both groups, a total of 1606 metabolites were detected by LC-MS with negative ion mode, 299 of which could be qualitatively matched by MS. In order to visualize group separation and find significantly changed metabolites, we applied supervised orthogonal projections to latent structures-discriminate analysis (OPLS-DA). As revealed by OPLS-DA, the differences between the two groups of samples were quite significant, and the samples were basically in the 95% confidence interval (CI) (Fig. 1a). Furthermore, we obtained the value of variable importance from the projection (VIP) of the first principal component in OPLS-DA. This summarized the contribution of each variable to the model. The metabolites with VIP>1 and P<0.05 were considered to be significantly changed metabolites. It is evident from the volcano plot that there were 104 higher metabolites (30 qualitative metabolites) and 162 lower metabolites (20 qualitative metabolites) in the AAA group (Fig. 1b).

The differential metabolites obtained from the above analysis often have similarity and complementarity functions in biology, or are positively or negatively regulated by the same metabolic pathway, showing similar or opposite expression characteristics between the AAA and AS groups. Hierarchical clustering analysis of these characteristics classified the metabolites with the same characteristics into one group (Fig. 1c). Then we classified the different metabolites, calculated the related metabolites by Spearman analysis, and finally visualized them with a chord plot. All 50 metabolites were classified as benzenoids, lipids and lipid-like molecules, organic acids and derivatives, organic compounds, organoheterocyclic compounds, organooxygen compounds, or others (Fig. 2). In addition, commercial databases including KEGG and MetaboAnalyst were used for pathway enrichment analysis. We found that cysteine and methionine metabolism was the most significant pathway after enrichment analysis (Fig. 3).

3.3 Filtration of differential metabolites between AAA and AS patients

In order to reduce the number of differential metabolites for further analysis, the fold change was set as >1.50 or <0.67. Eighteen metabolites were confirmed: cysteine-*S*-sulfate, mevalonic acid, 5-L-glutamyl-L-alanine, 3-methoxy-4-hydroxyphenylglycol sulfate, pregnenolone sulfate, glycochenodeoxycholate, 2'-deoxy-D-ribose (2dDR), glycocholic acid, and adynerin were expressed more highly in the AAA group, and nine other metabolites were increased in the AS group (Fig. 4, Table S1).

3.4 Subgroup analyses of differential metabolites in AAA patients

The threshold diameter for AAA repair is always set as 5.5 cm. To further examine the role of differential metabolites in AAA patients, we performed a subgroup comparative analysis between large (maximum diameter ⩾5.5 cm, n=13) and small (maximum diameter <5.5 cm, n=19) AAAs. As shown in Table 2, 12 significantly differential metabolites were identified. For both this group of metabolites and the 18 filtered differential metabolites found when comparing AAA and AS, 2dDR was the only overlapping one.

3.5 Diagnostic values of differential metabolites in AAA and AS patients

To test the diagnostic efficiencies of 18 selected metabolites between AAA and AS, an ROC was generated, and the AUC was computed for each incorporated feature (Fig. 5a). L-Proline had the highest AUC (0.76, 95% CI: 0.63–0.87) among all 18 metabolites, but was still insufficient to distinguish the two diseases.

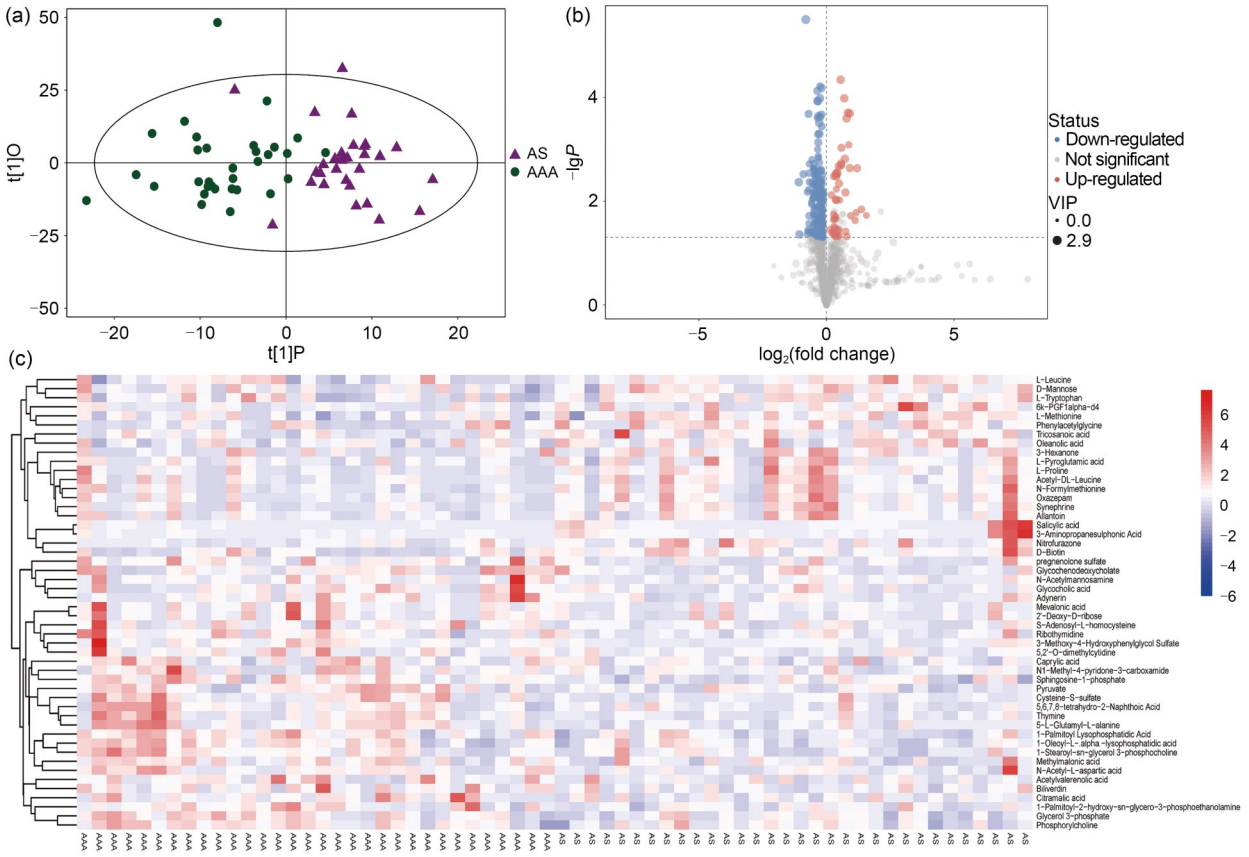


Fig. 1 Differential metabolites screening between AAA and AS patients. (a) The OPLS-DA showed that the samples are basically in the 95% CI, and AAA and AS patients can be clearly separated. (b) The volcano plot visually showed detected metabolites between the two groups. (c) The heatmap showed all 50 qualitative metabolites in the samples of two groups. AAA: abdominal aortic aneurysm; AS: atherosclerosis; VIP: variable importance from the projection; OPLS-DA: orthogonal projections to latent structures-discriminate analysis; CI: confidence interval.

With the linear SVM algorithm, the best biomarker model was obtained by combining synephrine, salicylic acid, pregnenolone sulfate, 5-L-glutamyl-L-alanine, 3-hexanone, and nitrofurazone with mevalonic acid, and the peak AUC of the combined model was significantly higher than that of the single-metabolite model (AUC: 0.93, 95% CI: 0.83–1.00; Fig. 5b).

3.6 DEG screening and integration analysis

We selected 49 AAA samples and 9 AS atheroma samples from the GEO dataset (GSE57691). The threshold for DEG screening was set as $|\log_2(\text{fold change})|>1$ and adjusted to $P<0.01$. In total, 75 DEGs were screened, of which 72 DEGs were up-regulated and 3 were down-regulated. The expression patterns of DEGs are shown in Fig. 6 and Table 3. The results of GO and KEGG pathway analyses are listed in Table S2. Spearman's correlation test was conducted between differential metabolites and DEGs to investigate the associations between two omics. Seven metabolites showed a correlation with transcriptomics, and the results were visualized as a correlation heatmap (Fig. 7). Furthermore, since GSE57691 also classified AAA samples into small AAA and large AAA, we conducted a subgroup analysis for AAA size integrating transcriptomics and metabolomics (Fig. S1).

4 Discussion

In this study, we performed a comprehensive untargeted metabolomic evaluation to explore the differences between AAA and AS in plasma metabolites, and further explored the associations between metabolites and expressed genes. First, we were able to identify a total of 18 differential metabolites between the two groups. Second, we discovered that the combination of synephrine, salicylic acid, pregnenolone sulfate,

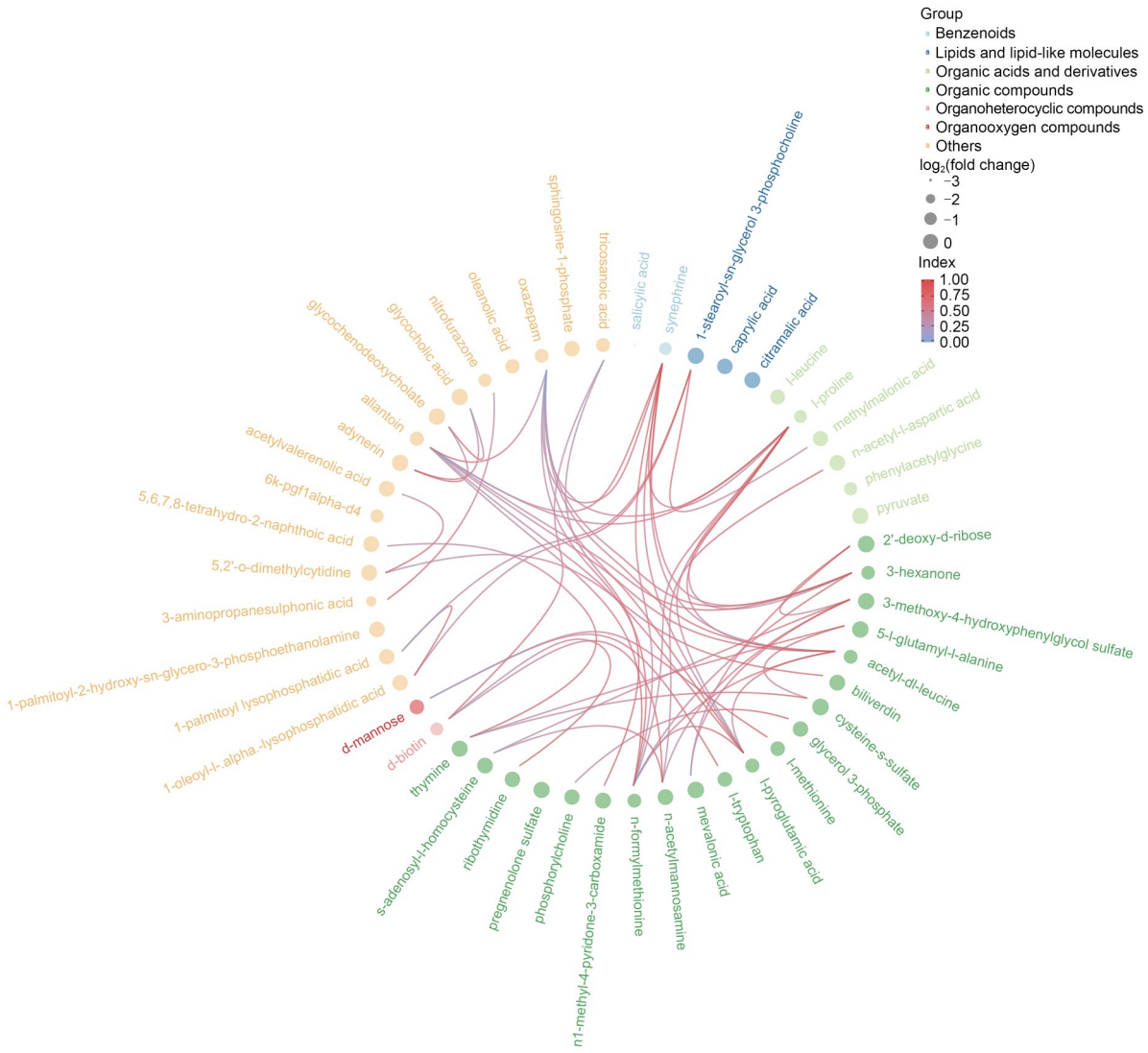


Fig. 2 Chord plot analysis. All 50 differential metabolites were attributed to seven categories.

5-L-glutamyl-L-alanine, 3-hexanone, nitrofurazone, and mevalonic acid could potentially serve as a biomarker for distinguishing between AAA and AS. Third, five metabolites including mevalonic acid, 3-methoxy-4-hydroxyphenylglycol sulfate, 2dDR, pregnenolone sulfate, and adynerin were found to be positively correlated with DEGs such as FK506-binding protein 5 (*FKBP5*), ribosomal protein L31 (*RPL31*), and *RPL26*.

AAA and AS are both complex multifactorial diseases with known environmental and genetic risk factors that contribute to disease development. On the one hand, AS represents an important independent risk factor for AAA, as patients with AAA often suffer from AS. The incidence of coronary artery disease in people with AAA was 53%, highlighting that AS is not likely to be a causal event in AAA (Ito et al., 2008). On the other hand, there are still some differences in clinical risk factors and deeper mechanisms between AAA and AS (Golledge and Norman, 2010). For example, diabetes is a risk factor which can lead to AS progressing, while some recent studies have observed that diabetes is independently associated with lower risk and small aortic diameter of AAA (Ning et al., 2020). Therefore, the relationships between AAA and AS remain unclear. More related studies from various perspectives and novel biomarkers with better clinical utility are needed. At present, AAA screening is mainly for men over 65 years old and offers lower cost effectiveness (US Preventive Services

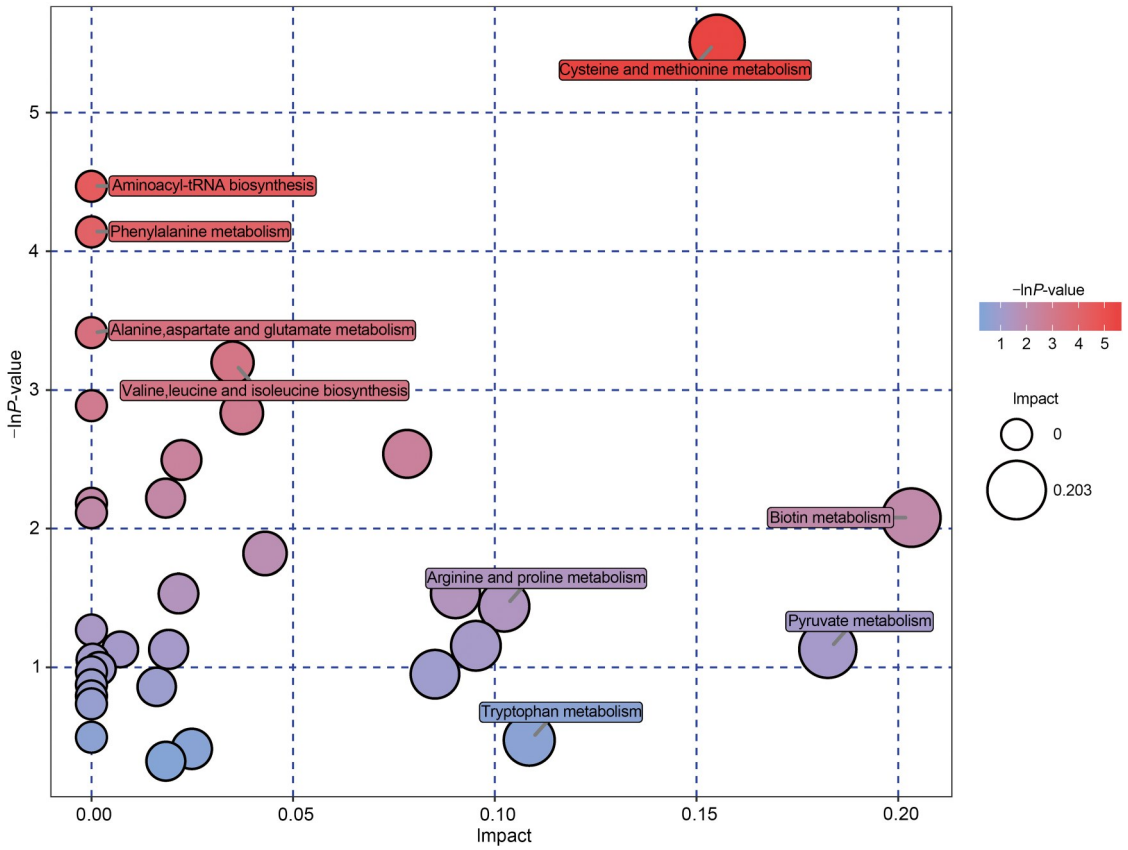


Fig. 3 Pathway enrichment analysis. Each bubble represents a metabolic pathway. The position at the abscissa and the size of the bubble represent the impact of the pathway. The position at the ordinate and the color of the bubble represent the enriched degree.

Task Force, 2019). It is valuable for disclosing the high-risk population for AAA in elderly patients with AS. In the current study, the AUC values were calculated for all identified metabolites using univariate ROC analyses. However, no metabolite was sensitive enough to be a biomarker, and the AUC values were between 0.60 and 0.76. Next, we manually selected combinations of metabolites to create biomarker models using linear SVM and found that a combination of synephrine, salicylic acid, pregnenolone sulfate, 5-L-glutamyl-L-alanine, 3-hexanone, and nitrofurazone with mevalonic acid could potentially serve as a biomarker to distinguish AAA and AS. It had an AUC of 0.93 and may set the stage for future research and clinical translation.

Among DEGs screened in transcriptomic analysis, *RPL31* and *RPL26* had the highest $\log_2$(fold change). *RPL31* and *RPL26* both belong to the ribosomal protein family. Gan et al. (2015) found that *RPL31* was up-regulated in patients with Takayasu arteritis. *RPL26* was found to play an important role in up-regulating *p53* expression (Takagi et al., 2005). Although the role of *p53* in aneurysm formation is not clear yet, Leeper et al. (2013) suggested that *p53*-dependent smooth muscle apoptosis might participate in AAA formation. In addition to an increase in *p53* signaling, they also found a reduction of transformed mouse 3T3 cell double minute 2 (*MDM2*) in AAA formation. Since *MDM2* was found to regulate *p53* expression by inhibitory interactions with *RPL26* (Ofir-Rosenfeld et al., 2008), we inferred that *MDM2-RPL26-p53* axis might play a role in AAA formation.

Correlated analysis of transcriptomics data showed that five metabolites (mevalonic acid, 3-methoxy-4-hydroxyphenylglycol sulfate, 2dDR, pregnenolone sulfate, and adynerin) were positively correlated with differentially expressed genes, which all increased in AAA. 2dDR had the second largest fold change (1.71) between AAA and AS and was the only overlapping filtered metabolite in subgroup analysis

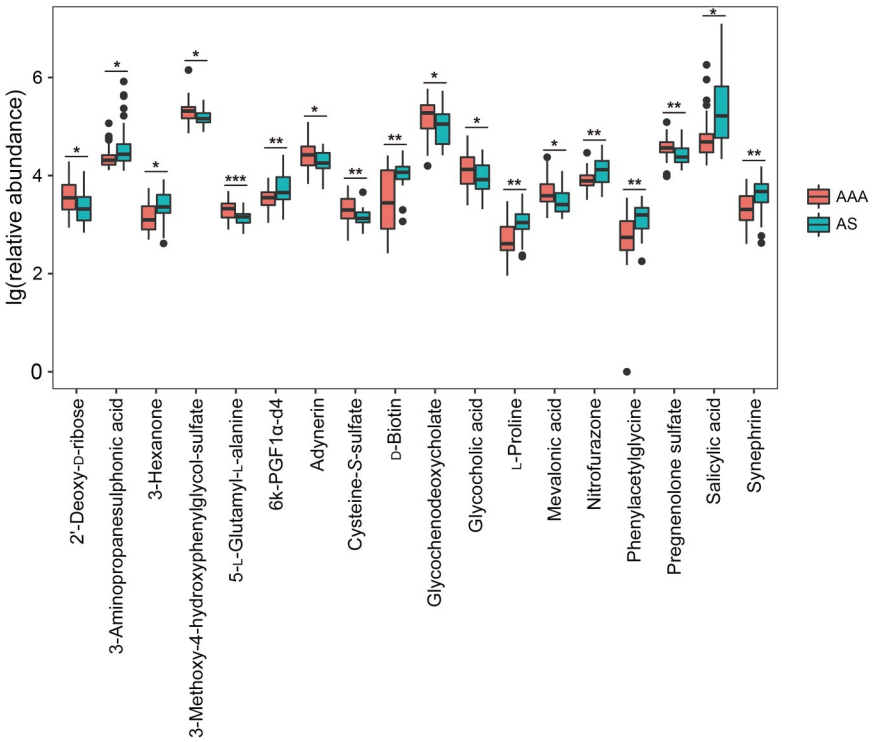


Fig. 4 Filtered differential metabolites between AAA and AS patients. * ***P*<0.05,** ** ***P*<0.01,** *** ***P*<0.001. AAA: abdominal aortic aneurysm; AS: atherosclerosis.**

Table 2 Differential metabolites between large and small AAAs

Metabolite	Mean		VIP	*P*-value	Fold change
	Large AAA	Small AAA			
2dDR	0.071 391 891	0.033 085 083	2.508 852 558	0.048 002 981	2.157 827 214
Tridecanoic acid	0.357 558 348	0.242 136 708	2.845 694 750	0.003 612 643	1.476 679 647
Cellobiose	0.012 745 954	0.008 677 840	2.135 654 099	0.029 007 197	1.468 793 321
Acetohydroxamic acid	0.028 123 177	0.020 150 806	1.759 087 615	0.042 008 168	1.395 635 331
D-Ribose	0.498 246 565	0.383 815 504	2.515 926 243	0.040 888 277	1.298 140 797
D-Threitol	0.388 525 175	0.445 448 059	1.612 862 776	0.047 210 139	0.872 212 073
L-Valine	1.501 790 518	1.725 737 775	2.053 685 057	0.021 940 332	0.870 231 005
D-Mannose	1.399 298 578	1.608 589 801	1.773 668 079	0.042 645 427	0.869 891 489
L-Tryptophan	1.189 142 462	1.410 047 883	2.275 400 929	0.019 603 593	0.843 334 809
L-Methionine	0.075 198 548	0.093 050 281	2.833 742 019	0.000 338 634	0.808 149 610
L-Leucine	2.837 439 159	3.565 975 188	2.651 520 606	0.004 620 671	0.795 697 954
3-Hydroxyisovaleric acid	0.246 970 576	0.514 297 551	1.370 013 092	0.041 108 243	0.480 209 512

AAA: abdominal aortic aneurysm; VIP: the value of variable importance from the projection; 2dDR: 2'-Deoxy-D-ribose.

(*P*=0.048), which deserves more attention. 2dDR is a deoxy-sugar derived from 2'-deoxy-D-1-phosphorylas, which also displays chemotactic activity in vitro (Haraguchi et al., 1994). A strong relationship between 2dDR and vascular endothelial growth factor (VEGF) was reported in a recent study (Dikici et al., 2020). VEGF and its receptors have a great pro-angiogenic effect through promoting the mobilization of inflammatory cells to pathological lesions, inducing the synthesis of pro-angiogenic factors by smooth muscle cells and more (Ferrara et al., 1991; Knox et al., 2001; Murakami et al., 2006; Ucuzian et al., 2010; van Hove and Benoit, 2015). Meanwhile, the significance of VEGF and angiogenesis in the development of AAA

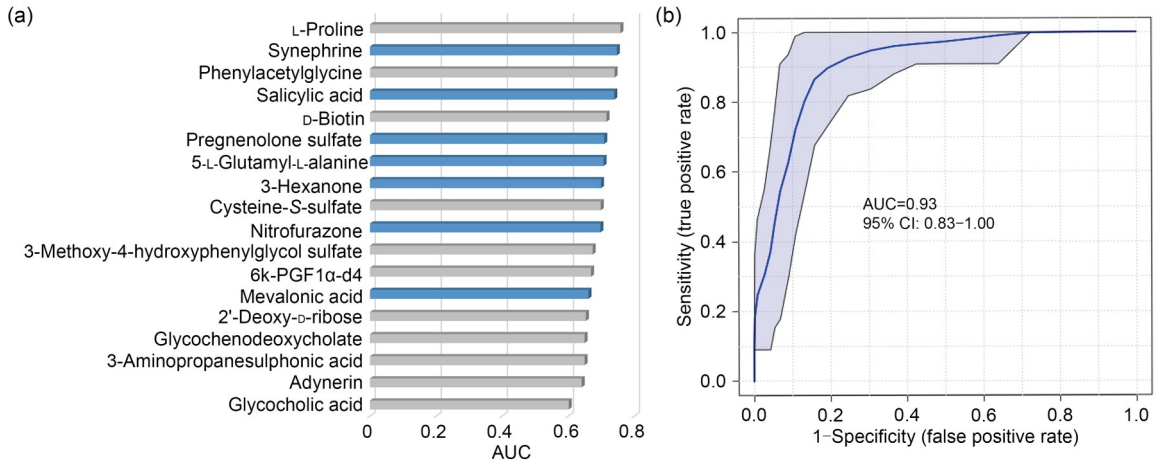


Fig. 5 ROC analysis for diagnostic values of differential metabolites. (a) Single variate analysis. Columns with blue color represent metabolites filtered by SVM algorithm. (b) Multivariate model using linear SVM algorithm (synephrine, salicylic acid, pregnenolone sulfate, 5-L-glutamyl-L-alanine, 3-hexanone, and nitrofurazone with mevalonic acid). AUC: area under the curve; ROC: receiver operator characteristic curve; SVM: support vector machine.

Table 3 Differentially expressed genes (DEGs) with top-20 $|\log_2(\text{fold change})|$

Gene symbol	Gene name	$\log_2$(fold change)	*P*-value
RPL31	Ribosomal protein L31	2.947 149	8.12×10^{-9}
RPL26	Ribosomal protein L26	2.841 221	0.020 029
MT1X	Metallothionein 1X	2.424 021	0.000 933
RPL24	Ribosomal protein L24	2.337 415	0.006 584
ITGA8	Integrin subunit α8	2.208 935	0.002 434
RPL39	Ribosomal protein L39	2.007 809	0.019 766
SERPINA3	Serpin family A member 3	1.626 579	0.006 732
RPS25	Ribosomal protein S25	1.554 147	0.007 467
C12orf57	Chromosome 12 open reading frame 57	1.523 047	0.000 357
TMEM14B	Transmembrane protein 14B	1.407 274	0.033 631
CCDC72	Translation machinery associated 7 homolog	1.341 165	0.001 206
PRRX2	Paired-related homeobox 2	1.337 391	2.48×10^{-6}
FKBP5	FKBP prolyl isomerase 5	1.325 069	0.000 432
NDUFS5	NADH:ubiquinone oxidoreductase subunit S5	1.307 802	0.018 787
RPS21	Ribosomal protein S21	1.271 324	0.007 063
NOL7	Nucleolar protein 7	1.258 512	0.031 010
ITGA10	Integrin subunit α10	1.253 124	0.000 379
ID3	Inhibitor of DNA binding 3, HLH protein	1.244 650	0.001 011
C14orf156	SRA stem-loop interacting RNA binding protein	1.230 766	0.042 435
FCN1	Ficolin 1	−1.211 590	0.044 670

FKBP: FK506-binding protein; NADH: nicotinamide adenine dinucleotide; HLH: helix-loop-helix; SRA: steroid receptor RNA activator protein.

has already been confirmed. Pharmacological inhibition of VEGF substantially mitigates both AAA formation and further progression of established AAA in mice (Xu et al., 2019). In addition, 2dDR can induce cell apoptosis by provoking oxidative stress and reducing glutathione depletion (Ardestani et al., 2008; Fico et al., 2008). On the other hand, through integration analysis with transcriptomics data, FKBP5, a protein implicated in stress physiology, has been found to be positively correlated with 2dDR. A recent study revealed that upregulation of FKBP5 promotes nuclear factor-κB (NF-κB) signaling, which is a critical pro-inflammatory

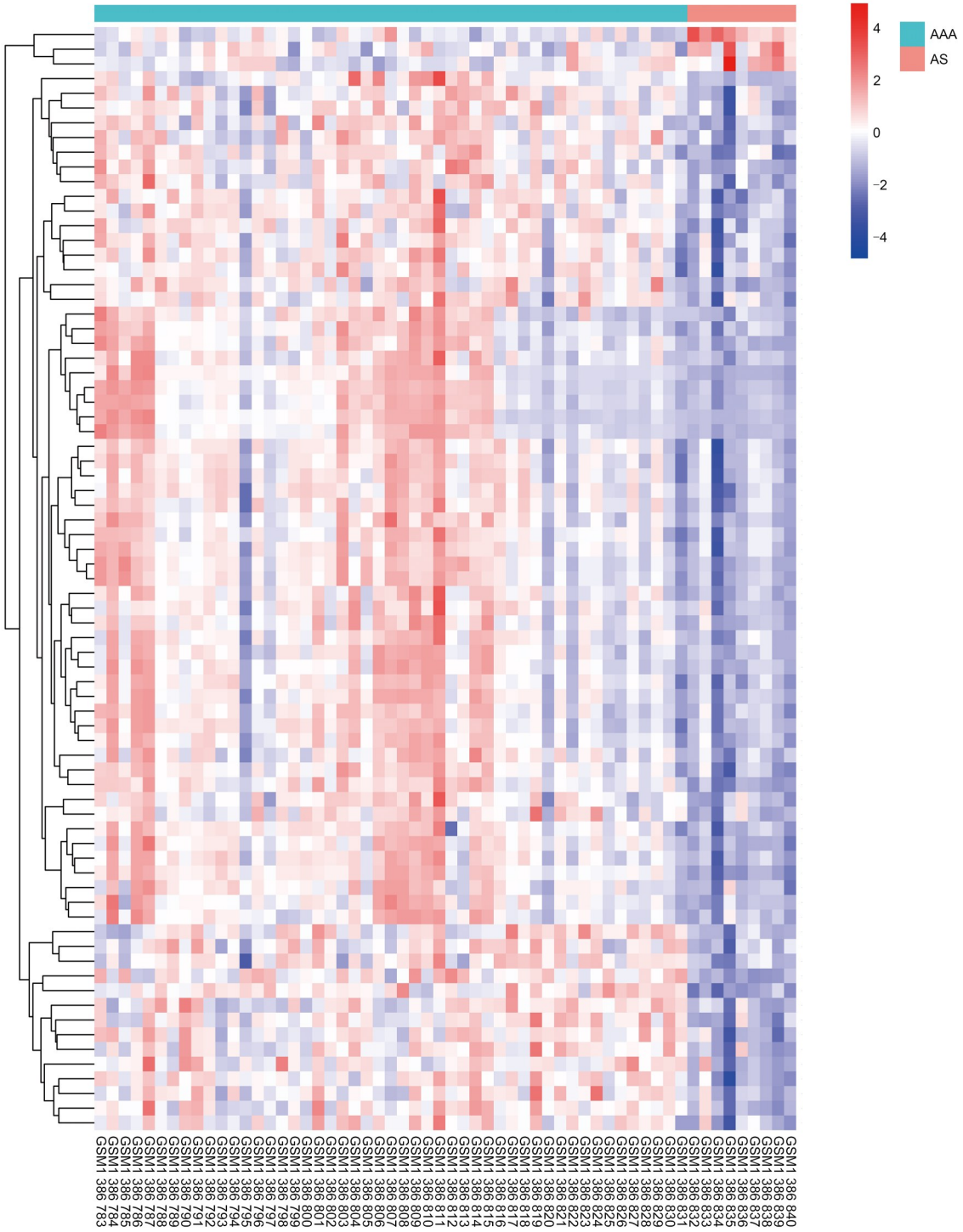


Fig. 6 Heatmap showed DEGs between AAA and AS patients. AAA: abdominal aortic aneurysm; AS: atherosclerosis; DEG: differentially expressed gene.

factor in AAA (Ma et al., 2018), by strengthening the interaction of key regulatory kinases, and NF-κB binding to the FKBP5 enhancer can in turn stimulate FKBP5 expression. The positive feedback loop eventually contributes to inflammation and cardiovascular risk (Zannas et al., 2019). It is plausible to hypothesize that 2dDR may promote the formation of AAA via angiogenesis and inflammation induced by upregulation

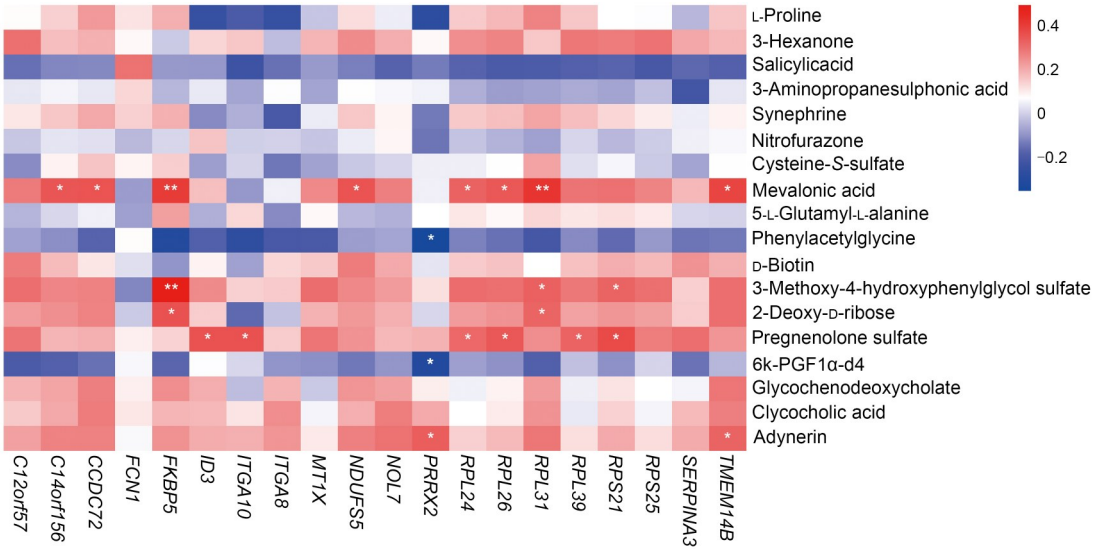


Fig. 7 Correlation analysis combined differential metabolites with expressed genes. * ***P*<0.05,** ** ***P*<0.01.**

of VEGF and NF-κB, as well as apoptosis of some key cells.

As this is a pilot study, there are several limitations in our work. First, the transcriptomics and metabolomics data were from different patients and samples. The integration analysis of the data for these two omics data can only be exploratory, because paired analysis using samples from the same patients would be more optimal. Second, the enrolled patients were from a single Chinese center, which had obvious regional characteristics. Third, in order to ensure consistency of the samples, all patients enrolled in the AS group suffered from carotid artery stenosis, and thus could not fully represent AS. Fourth, there are various factors that may have affected the plasma metabolites, such as diet and environment, and the AAA patients were also older than the AS patients ((68.6±5.7) vs. (64.5±6.7) years, P=0.019). We found that aging was associated with altered plasma triglyceride metabolism, for example in the mammalian target of rapamycin (mTOR) and adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathways (Sharma and Ramanathan, 2020; Spitler and Davies, 2020) and our study did not take all these potential variations into consideration. Last but most important, the results need to be verified with a larger independent cohort of patients, the metabolites should be confirmed with their chemical standards, and all findings should be further confirmed by experimental models.

5 Conclusions

We carried out an untargeted metabolomic study with plasma samples from patients with AAA and AS, achieving identification of 18 metabolites that could be different in the progression of these two diseases. Filtered metabolites accurately distinguished AAA from AS with an AUC value of 0.93. In addition, combined with transcriptomics and a review of the literature, we found that 2dDR may be an important pathogenetic metabolite in the development of atherosclerotic AAA, which merits further research.

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Author contributions

Lei JI and Siliang CHEN performed the experimental research and data analysis, wrote and edited the manuscript. Guangchao GU, Wei WANG, Jinrui Ren, Fang XU, Fangda LI, and Jianqiang WU collected the samples. Yuehong ZHENG, Dan YANG, Lei JI, and Siliang CHEN contributed to the study design, data analysis, writing and editing of the manuscript. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Lei JI, Siliang CHEN, Guangchao GU, Wei WANG, Jinrui REN, Fang XU, Fangda LI, Jianqiang WU, Dan YANG, and Yuehong ZHENG declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975; as revised in 2008 (5). This study was approved by the Ethics Committee of the Peking Union Medical College Hospital, China (JS-2629). Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

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Supplementary information

Tables S1 and S2; Fig. S1