

基于非靶向代谢组学方法探索代谢相关脂肪性肝病患者 亚临床动脉粥样硬化的潜在代谢标志物

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[摘要] 目的: 代谢相关脂肪性肝病(metabolic dysfunction-associated fatty liver disease, MAFLD)最常见的肝外并发症及死因是心血管疾病, MAFLD患者发生亚临床动脉粥样硬化(subclinical atherosclerosis, SA)的早期代谢标志物目前尚不明确。本研究通过非靶向代谢组学探索MAFLD患者发生SA的血清代谢物及代谢途径的变化, 筛选潜在的血清代谢标志物。方法: 选取2023年12月至2024年12月在中南大学湘雅三医院健康管理医学中心“泛血管队列研究”纳入的64例MAFLD合并SA患者作为实验组, 同期选取50例年龄、性别匹配的MAFLD未合并SA的受检者作为对照组。MAFLD诊断标准参照《代谢相关(非酒精性)脂肪性肝病防治指南(2024年版)》, 采用多血管节段动脉粥样硬化斑块定义SA。使用超高效液相色谱和质谱仪进行非靶向代谢组学检测。采用差异倍数(fold change, FC)分析、主成分分析和正交偏最小二乘判别分析方法筛选差异代谢物, 受试者操作特征(receiver operating characteristic, ROC)曲线评价差异代谢物的诊断效能, 通路富集分析方法筛选与MAFLD及动脉粥样硬化发生相关的通路。结果: 基于差异代谢物筛选标准错误发现率(false discovery rate, FDR)-corrected $q < 0.05$, $FC > 1.5$ 或 < 0.67 , 2组间共筛选出415个差异代谢物。排名前5的差异代谢物为溶血磷脂酰胆碱(18:3)、白屈菜红碱、N,N-六亚甲基硫代氨基酸-S-乙酯、N-乙基-邻巴豆酰甲基苯胺、溶血磷脂酰胆碱(18:1)。选取这5个差异代谢物使用支持向量机(support vector machine, SVM)进行建模并绘制ROC曲线, 曲线下面积为1。差异代谢物主要富集于组氨酸代谢、鞘脂代谢、甘油磷脂代谢、不饱和脂肪酸生物合成、色氨酸代谢、牛磺酸与亚牛磺酸代谢等通路。结论: MAFLD患者是否发生SA的代谢谱存在显著差异, 关键差异代谢物可能是预测MAFLD患者是否发生动脉粥样硬化的潜在生物标志物。

[关键词] 代谢相关脂肪性肝病; 亚临床动脉粥样硬化; 代谢组学; 溶血磷脂酰胆碱; 生物标志物

Untargeted metabolomics-based exploration of potential metabolic biomarkers for subclinical atherosclerosis in metabolic-associated fatty liver disease

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ABSTRACT

Objective: Cardiovascular disease is the most common extrahepatic complication and leading cause of death in metabolic dysfunction-associated fatty liver disease (MAFLD). However, early metabolic biomarkers for subclinical atherosclerosis (SA) in MAFLD patients remain unclear. This study used untargeted metabolomics to investigate serum metabolite and pathway changes associated with SA in MAFLD patients and to screen for potential serum metabolic biomarkers.

Methods: From December 2023 to December 2024, 64 MAFLD patients with SA enrolled in the “Pan-Vascular Cohort Study” at the Health Management Medical Center of the Third Xiangya Hospital of Central South University were included as an experimental group, and 50 age- and sex-matched MAFLD patients without SA were included as controls. MAFLD was diagnosed according to the *Guidelines for the Prevention and Treatment of Metabolic (Non-Alcoholic) Fatty Liver Disease (2024 Edition)*. SA was defined by the presence of multi-segment arterial plaques and/or peripheral arterial obstruction. Untargeted metabolomics profiling was performed using ultra-high performance liquid chromatography coupled with mass spectrometry. Differential metabolites were screened using fold change (FC) analysis, principal component analysis, and orthogonal partial least squares discriminant analysis. Receiver operating characteristic (ROC) curves evaluated diagnostic performance of differential metabolites, and pathway enrichment analysis identified metabolic pathways associated with MAFLD and atherosclerosis.

Results: Using selection criteria of FDR-corrected $q < 0.05$ and $FC > 1.5$ or < 0.67 , a total of 415 differential metabolites were identified between the 2 groups. The top 5 differential metabolites were lysophosphatidylcholine (18 : 3), chelerythrine, N, N-hexamethylene thiocarbamate S-ethyl ester, N-ethyl-o-crotonylmethylaniline, and lysophosphatidylcholine (18 : 1). ROC analysis based on support vector machine (SVM) modeling of these top 5 metabolites yielded an area under the curve of 1. Differential metabolites were mainly enriched in histidine metabolism, sphingolipid metabolism, glycerophospholipid metabolism, unsaturated fatty acid biosynthesis, tryptophan metabolism, and taurine/hypotaurine metabolism.

Conclusion: The metabolic profiles of MAFLD patients with and without SA differ significantly. Key differential metabolites may serve as potential biomarkers for predicting atherosclerosis in MAFLD patients.

KEY WORDS

metabolic dysfunction-associated fatty liver disease; subclinical atherosclerosis; metabolomics; lysophosphatidylcholine; biomarker

非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD)是常见的慢性肝病,包括从单纯脂肪变性到非酒精性脂肪性肝炎(non-alcoholic steatohepatitis, NASH)、肝硬化,甚至发展成需要肝移植的终末期肝病,肝脏脂肪沉积是其主要特征^[1]。它不是一种孤立的疾病,而是一种合并其他代谢因素的疾病。目前认为NAFLD是代谢综合征的肝脏表现^[2]。2020年3月,国际专家小组提出以新命名“代谢相关脂肪性肝病(metabolic dysfunction-associated fatty liver disease, MAFLD)”取代NAFLD,这一命名旨在强调代谢异常在其病理生理学机制中的作用^[3]。MAFLD目前已成为影响全球1/3成年人口的第一大慢性肝病,中国MAFLD患病率为29.7%,发病率为56.4/1 000人年,二者都随着年龄增加而升高。MAFLD已成为中国日益严峻的公共卫生问题^[4-5]。

MAFLD作为一种多系统受累的代谢性疾病,主要死因依次为动脉粥样硬化性心血管疾病(atherosclerotic cardiovascular disease, ASCVD)、非肝脏的恶性肿瘤及肝脏相关并发症^[6-7]。MAFLD与心血管疾病关系极为密切,它们可能共享类似的发病机制,如胰岛素抵抗、脂肪酸及甘油三酯代谢异常、慢性炎症、内皮功能障碍、氧化应激等^[8-13]。2023年《MAFLD与心血管疾病风险国际多学科专家共识》^[14]提出MAFLD为一种新的ASCVD危险因素,但仍然未得到充分的认识和关注。

亚临床动脉粥样硬化(subclinical atherosclerosis, SA)虽不表现出典型的临床症状,但却是发展成急性缺血事件的关键中间环节,对心血管健康构成潜在的严重威胁^[15]。在严重ASCVD事件发生前,聚焦ASCVD早期病变(动脉粥样硬化进展)筛查与干预,对降低远期ASCVD负担具有重要意义^[16]。MAFLD已被证实与SA相关指标(包括较高的颈动脉内膜中层厚度、较大的冠状动脉钙化斑块、冠状动脉高危阻塞性斑块和非钙化斑块)存在联系^[17]。

聚焦MAFLD患者的SA病变,筛选血清标志物,进行早期风险预警,适应了重大疾病防控“关口前移”的需求,具有非常重要的临床和公共卫生应用价值^[18]。近年来,代谢组学技术的发展为揭示ASCVD复杂的病因学机制带来了前所未有的机遇。代谢组学可以全面评估人体代谢物与心血管代谢疾病的关联,识别关键代谢标志物和代谢途径,为疾病的风险评估提供了强有力的工具^[19-20]。因此,本研究旨在通过非靶向代谢组学探索MAFLD患者发生SA的血清代谢物及代谢途径的变化,并筛选潜在的血清代谢标志物,为后期进一步探寻干预靶点提供支撑。

1 对象与方法

1.1 伦理声明

本研究获得中南大学湘雅三医院伦理委员会批准(审批号:2023-S332),所有参与者均签署了知情同意书。

1.2 对象

选取2023年12月至2024年12月在中南大学湘雅三医院健康管理医学中心纳入“泛血管队列研究”的64例MAFLD合并SA患者作为实验组,同期选取50例年龄、性别匹配的MAFLD未合并SA患者作为对照组。纳入标准:年龄为40~70岁,既往无心血管疾病(cardiovascular disease, CVD)病史且诊断为MAFLD。排除标准:1)心脑血管疾病史(如心肌梗死、脑梗死、脑出血、痴呆等);2)恶性肿瘤病史;3)慢性肾病或肝硬化病史;4)多区域血管彩色多普勒超声(以下简称“彩超”)不全;5)缺乏人口学信息或实验室检查数据;6)不愿意参加本研究。

1.3 方法

1.3.1 基线资料收集

问卷采用中华医学会健康管理学分会统一编制的《健康体检自测问卷》,收集人口学信息、社会经济状况、疾病史、健康相关行为、家族史等。所有参与者均完成腰围、体重指数、血压、心电图、肝脏彩超及多血管节段彩超(颈动脉、髂动脉、股动脉和腹主动脉)检查。

1.3.2 生物样本采集和处理

采集所有参与者禁食8 h后的清晨空腹血样本,完成相关血液指标检测,包括血常规、肝功能、肾功能、血脂全套、空腹血糖、糖化血红蛋白、空腹胰岛素、乙丙型肝炎病毒标志物等。采集空腹血标本10 mL置于血清分离管,并在室温下凝固60 min,凝固结束后30 min内离心10 min(1 600×g, 4 °C)以去除不溶性固体,收集每份血清样本并立即储存于-80 °C冰箱,后续用于代谢组学检测。

1.3.3 MAFLD诊断标准

MAFLD诊断标准参照《代谢相关(非酒精性)脂肪性肝病防治指南(2024年版)》^[21],具体为:基于成年人肝脏脂肪变性的证据(符合肝脏超声),排除过量饮酒和可以导致脂肪肝的其他原因(如药物性脂肪肝、肝豆状核变性和营养不良等致脂肪肝的疾病),并且至少存在以下1项代谢综合征(metabolic syndrome, MetS)组分,1)超重/肥胖,体重指数 ≥ 24.0 kg/m²,或者男性腰围 ≥ 90 cm和女性 ≥ 85 cm,或者体脂肪含量

和体脂百分比超标, 或者男性腰围 ≥ 90 cm和女性 ≥ 80 cm; 2) 动脉血压增高/高血压病, 动脉血压 $\geq 130/85$ mmHg(1 mmHg=0.133 kPa), 或者在接受降血压药物治疗; 3) 糖尿病前期或2型糖尿病, 空腹血糖 ≥ 6.1 mmol/L, 或者糖负荷后2 h血糖 ≥ 7.8 mmol/L或糖化血红蛋白A1c(glycated hemoglobin A1c, HbA1c) $\geq 5.7\%$, 或者有2型糖尿病史, 或者稳态模型评估的胰岛素抵抗指数(homeostatic model assessment of insulin resistance, HOMA-IR) ≥ 2.5 ; 4) 血浆甘油三酯(triglyceride, TG)升高, 空腹TG ≥ 1.7 mmol/L, 或者正在接受降血脂药物治疗; 5) 血液高密度脂蛋白胆固醇下降, 男性血浆高密度脂蛋白胆固醇 < 1.0 mmol/L和女性 < 1.3 mmol/L或接受特异性药物治疗。

1.3.4 SA界定标准

采用西门子Sequoia 512超声诊断系统进行动脉粥样硬化斑块检测。检测部位包括4个血管区域节段, 分别为双侧颈动脉(颈总动脉和颈动脉分叉部)、双侧锁骨下动脉、腹主动脉和髂股动脉(髂总动脉和双侧髂内外动脉近端)。检查前嘱参与者采取仰卧位, 静息10 min, 将检查部位完全暴露, 依据《血管超声检查指南》^[22]诊断动脉斑块及评估各个部位斑块的数量。SA定义为局部隆起突出于动脉管腔 > 0.5 mm或超过环绕内中膜厚度(intima-media thickness, IMT)的50%或IMT > 1.5 mm。每个血管区域如存在至少1个斑块者, 定义为斑块受累区域, 且左右双侧血管计算为1个受累部位。为提高SA的区分度, 研究将至少2个区域血管节段均存在血管斑块者定义为多血管区域SA, 将0个区域血管节段存在血管斑块者定义为无SA^[23]。

1.3.5 代谢组学检测

2组血清样本于4℃解冻, 每例样本取100 μ L, 加入300 μ L预冷的甲醇, -20℃静置30 min, 16 000 $\times g$ 4℃离心20 min, 取上清, 后续用于质谱分析。整个分析过程中样品置于4℃自动进样器中, 采用SHIMADZU-LC30超高效液相色谱系统(ultra-high performance liquid chromatography, UHPLC)和ACQUITY UPLC[®] HSS T3(2.1 $\times$ 100 mm, 1.8 μ m)(Waters, Milford, MA, USA)色谱柱。每例样品分别采用电喷雾电离(electrospray ionization, ESI)进行正离子和负离子模式检测。样品经超高效液相色谱(ultra-performance liquid chromatography, UPLC)分离后用Orbitrap Fusion Lumos Tribrid质谱仪(Thermo Scientific)进行质谱分析, 使用加热电喷雾电离(heated electrospray ionization, HESI)源进行离子化, 最后进行质谱采集。

1.3.6 代谢组学数据处理及分析

原始数据采用MSDIAL软件进行峰对齐、保留时间校正和峰面积提取。代谢物结构鉴定采用精确质量数匹配(质量偏差 < 10 ppm)和二级谱图匹配(质量偏差 < 0.01 D)的方式, 检索人类代谢组数据库(Human Metabolome Database, HMDB)、MassBank、全球天然产物社会分子网络(Global Natural Products Social Molecular Networking, GNPS)等公共数据库及自建的拜谱代谢物库。对提取得到的数据, 删除组内缺失值大于50%的离子峰; 对正负离子数据分别进行总峰面积归一化, 整合正负离子峰并应用Python软件进行模式识别, 数据经单位方差标度(unit variance scaling, UV)预处理后, 进行无监督模式下主成分分析(principal component analysis, PCA)以评估数据整体稳定性。

采用有监督模式下的正交偏最小二乘判别分析(orthogonal projections to latent structures-discriminant analysis, OPLS-DA)评价组间的整体代谢变化, 计算每个变量在第一主成分上的投影重要度(variable importance in projection, VIP), 通过置换试验交叉验证OPLS-DA模型是否存在过度拟合风险; 再以 $P < 0.05$ (Student's *t* 检验)和VIP > 1 为标准, 筛选组间差异代谢物。

利用京都基因和基因组数据库(Kyoto Encyclopedia of Genes and Genomes, KEGG), 对有KEGG注释的代谢物进行通路分析。使用支持向量机(support vector machine, SVM)进行建模, 通过受试者操作特征(receiver operating characteristic, ROC)曲线及曲线下面积(area under the curve, AUC)评价代谢物的鉴别能力。

为计算代谢物的截断值, 分别对排名前5的差异代谢物进行单变量ROC分析, 并找出其最佳截断值: 使用pROC包中的roc()函数构建模型, 以Youden指数最大化作为统计标准, 自动计算出上述5种代谢物有效区分对照组与实验组的最佳阈值。

1.4 统计学处理

采用SPSS 26.0统计学软件对数据进行分析。符合正态分布的连续变量以均数 $\pm$ 标准差($\bar{x} \pm s$)表示, 2组比较采用*t*检验; 非正态分布的连续变量以中位数(第1四分位数, 第3四分位数)表示, 组间比较采用Mann-Whitney检验。分类变量以频数(率)表示, 组间比较采用 χ^2 检验。检验水准 $\alpha = 0.05$, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 基线资料及相关血液指标比较

2组患者的体重指数、腰围和体脂率等体重体型

指标, 血压、血糖、血脂等心血管危险因素指标, 胆红素、转氨酶、白蛋白等反映肝脏代谢、合成能力及肾功能指标比较, 差异均无统计学意义(均 $P>0.05$, 表1)。

表1 MAFLD合并SA与MAFLD未合并SA的基线特征比较

Table 1 Comparison of baseline characteristics between MAFLD patients with SA and MAFLD patients without SA

组别	<i>n</i>	年龄/岁	男性/[例(%)]	体重指数/(kg·m ⁻²)	腰围/cm	体脂率/%
实验组	64	56.33±5.41	43(67.2)	25.51±2.73	88.72±7.92	27.61±5.78
对照组	50	54.75±4.20	30(60.0)	25.92±2.45	87.78±7.80	30.47±6.89
<i>t/χ²/Z</i>		-1.717	0.856	0.842	-0.621	0.874
<i>P</i>		0.101	0.364	0.402	0.919	0.323
组别	高血压病/[例(%)]	收缩压/mmHg	舒张压/mmHg	空腹血糖/(mmol·L ⁻¹)	糖化血红蛋白/%	胰岛素抵抗指数
实验组	19(29.7)	126.43±14.21	77.58±10.78	6.18±2.26	6.25±1.17	2.77(2.02, 3.69)
对照组	15(30.0)	121.61±13.06	77.27±8.99	5.66±1.78	5.92±0.70	2.66(2.19, 3.34)
<i>t/χ²/Z</i>	0.040	-1.827	-0.165	-1.296	-1.708	-0.528
<i>P</i>	0.841	0.571	0.402	0.378	0.063	0.601
组别	糖尿病/[例(%)]	甘油三酯/(mmol·L ⁻¹)	低密度脂蛋白胆固醇/(mmol·L ⁻¹)	高密度脂蛋白胆固醇/(mmol·L ⁻¹)	脂蛋白α/(nmol·L ⁻¹)	谷丙转氨酶/(U·L ⁻¹)
实验组	15(23.7)	1.92(1.35, 2.84)	2.87±0.78	1.23±0.25	72.0(55.0, 183.0)	22.0(17.0, 28.0)
对照组	7(14.0)	1.71(1.36, 2.59)	2.93±0.62	1.28±0.23	65.0(36.0, 134.0)	22.0(18.0, 29.0)
<i>t/χ²/Z</i>	2.077	-0.682	0.463	1.175	-1.225	-0.488
<i>P</i>	0.152	0.499	0.141	0.590	0.221	0.627
组别	谷草转氨酶/(U·L ⁻¹)	总胆红素/(μmol·L ⁻¹)	血清总蛋白/(g·L ⁻¹)	血清白蛋白/(g·L ⁻¹)	尿素氮/(mmol·L ⁻¹)	血肌酐/(mmol·L ⁻¹)
实验组	22.0(18.0, 25.0)	13.25±5.08	69.93±3.87	44.46±2.61	5.71±1.73	66.5(55.0, 77.8)
对照组	23.0(20.0, 27.0)	13.57±4.32	70.90±3.45	45.38±2.18	5.45±1.35	64.0(54.0, 83.0)
<i>t/χ²/Z</i>	-1.365	0.355	1.372	1.962	-0.867	-0.119
<i>P</i>	0.171	0.602	0.481	0.112	0.388	0.912

正态分布的连续变量以均数±标准差表示, 比较采用 *t* 检验; 非正态分布的连续变量以中位数(第1四分位数, 第3四分位数)表示, 比较采用 Mann-Whitney 检验。分类变量以频数(率)表示, 组间比较采用 χ^2 检验。1 mmHg=0.133 kPa。MAFLD: 代谢相关脂肪性肝病; SA: 亚临床动脉粥样硬化。

2.2 代谢组学数据质控

利用无监督模式下的PCA评估数据的整体稳定性, PCA得分结果(图1)提示聚集性良好, 数据质量较高。

2.3 代谢组学数据分析结果

分别采用PCA与OPLS-DA评价实验组与对照组之间的整体代谢变化。PCA结果显示, 2组之间呈现初步分离趋势(图2A); OPLS-DA结果显示, 2组之间呈现显著的分离趋势, 且模型未发生过拟合(图

2B)。上述结果提示, 2组之间代谢物组成存在显著差异。

2.4 差异代谢物的筛选及分析结果

对2组间代谢物进行Student's *t* 检验, 并计算差异倍数(fold change, FC)。差异代谢物筛选条件为: 错误发现率(false discovery rate, FDR)-corrected $q<0.05$, $FC>1.5$ 或 <0.67 。基于该筛选标准, 在实验组与对照组间共筛选出415个代谢物, 组间差异代谢物火山图见图3A, 排名前25的差异代谢物组间对比热图见图3B。

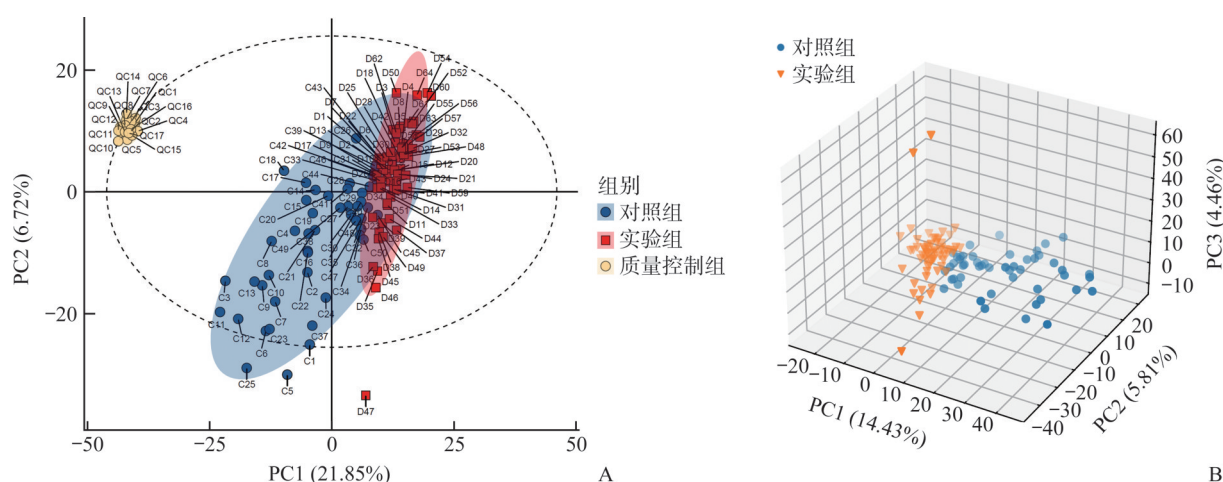


图1 实验组与对照组代谢组学数据质控图

Figure 1 Quality control plot of metabolomics data for experimental and control groups

A: Quality control plot of metabolomics data for the quality control, experimental, and health control groups. B: Quality control plot of metabolomics data for the experimental and health control groups. PC: Principal component.

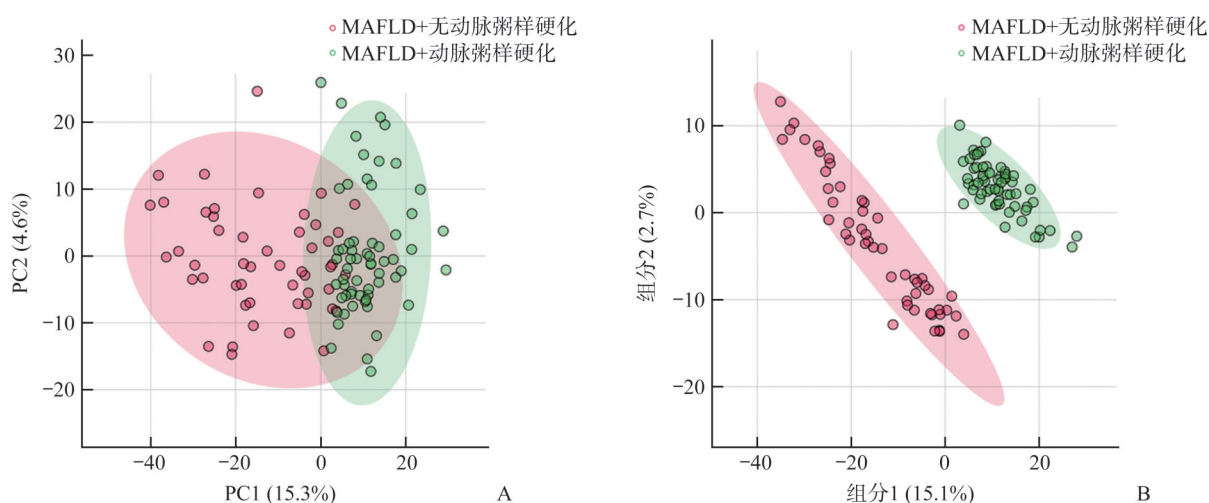


图2 实验组与对照组的PCA与OPLS-DA得分图

Figure 2 PCA and OPLS-DA score plots of experimental and control groups

A: PCA scores plot. B: OPLS-DA scores plot. PCA: Principal component analysis; OPLS-DA: Orthogonal partial least squares discriminant analysis.

2.5 差异代谢物的代谢通路分析结果

使用KEGG数据库对差异代谢物进行通路富集分析, 并使用气泡图对显著富集的排名前25的代谢通路进行展示。结果显示, 2组间的差异代谢物主要富集于氨基酸代谢、鞘脂代谢、甘油磷脂代谢、不饱和脂肪酸的生物合成、色氨酸代谢、牛磺酸与亚牛磺酸代谢等通路(图3C)。使用随机森林对上述组间差异代谢物进行重要性排序, 并对排名前15的代谢物进行展示(图4)。

排名前5的代谢物分别是溶血磷脂酰胆碱 (lysophosphatidylcholine, LPC)(18:3)、白屈菜红碱

(chelerythrine)、N,N-六亚甲基硫代氨基甲酸-S-乙酯 (molinate)、N-乙基-邻巴豆酰甲基苯胺(crotamiton)、LPC(18:1)。对排名前5的差异代谢物分别及联合使用SVM进行建模并绘制ROC曲线(图5A)。LPC(18:3)的AUC为0.979, chelerythrine的AUC为0.982, molinate的AUC为0.958, crotamiton的AUC为0.958, LPC(18:1)的AUC为0.938, 上述5种代谢产物的联合AUC为1。使用单变量ROC分析计算上述5种代谢物的截断值, 分别为7 745 965.985、2 991 696.705、564 324.330、94 090 924.220 和336 464 477.250(图5B~5F)。

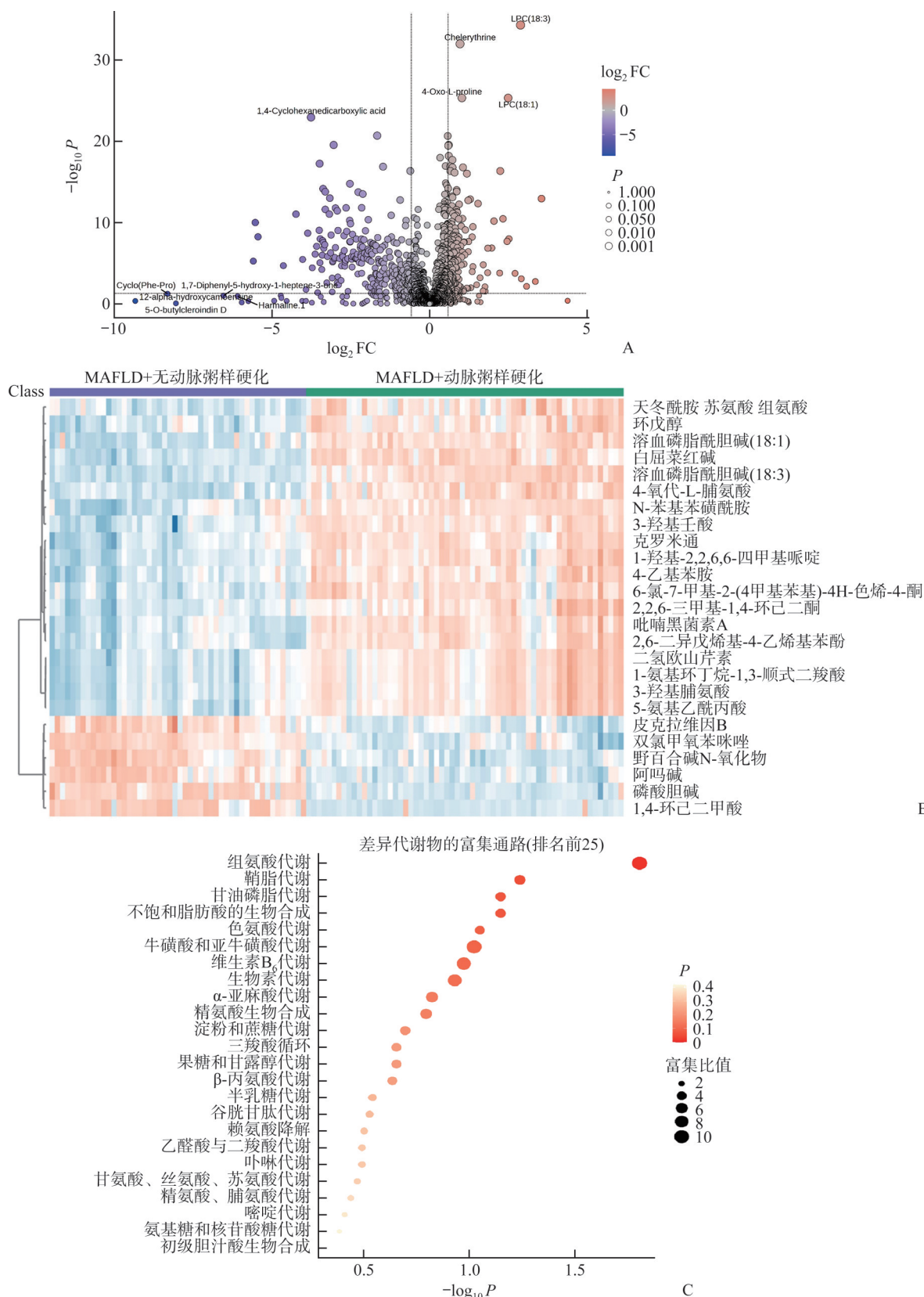


图3 实验组与对照组差异代谢物的筛选及分析结果

Figure 3 Screening and analysis results of differential metabolites in the experimental and control groups

A: Volcano plot of differential metabolite screening between experimental and control groups. B: Heatmap of the top 25 differential metabolites between experimental and control groups. C: Bubble chart of KEGG pathway enrichment analysis for differential metabolites between experimental and control groups. FC: Fold change.

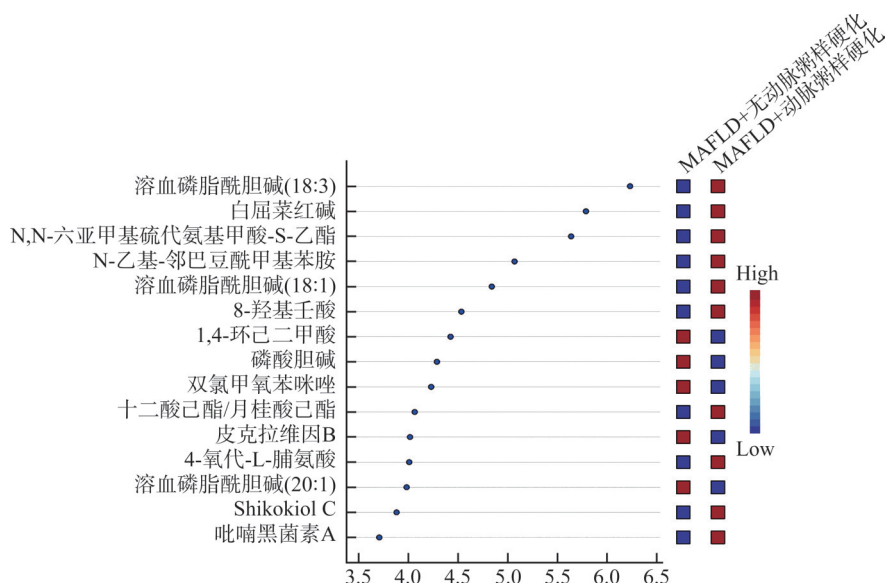


图4 随机森林分析筛选出的实验组与对照组间重要性排名前15的差异代谢物

Figure 4 Top 15 differential metabolites ranked by importance in random forest analysis between experimental and control groups

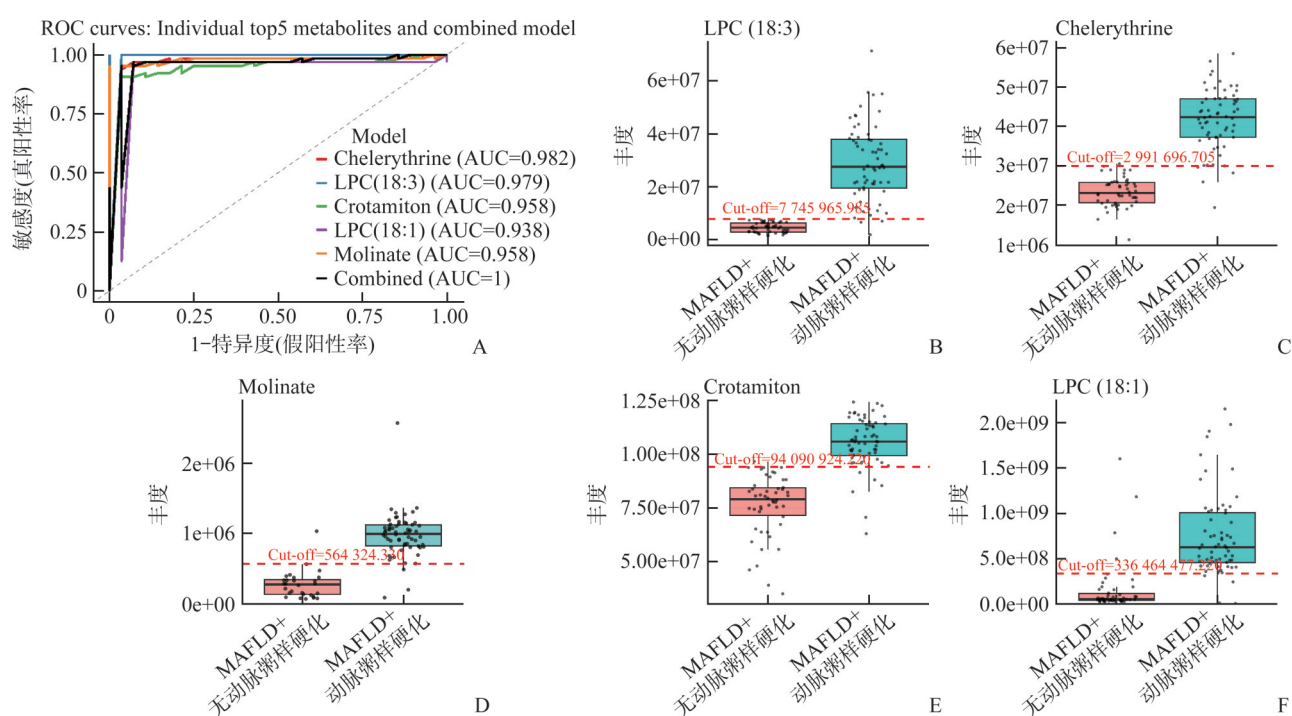


图5 排名前5的差异代谢物使用SVM模型绘制ROC曲线及单变量ROC分析截断值

Figure 5 ROC curves plotted using SVM model for the top 5 differential metabolites and the cut-off values from univariate ROC analysis

A: ROC curves plotted using SVM model for the top 5 differential metabolites. B: Cut-off value for the differential metabolite LPC (18:3) calculated by univariate ROC analysis. C: Cut-off value for the differential metabolite chelerythrine calculated by univariate ROC analysis. D: Cut-off value for the differential metabolite molinate calculated by univariate ROC analysis. E: Cut-off value for the differential metabolite crodamiton calculated by univariate ROC analysis. F: Cut-off value for the differential metabolite LPC (18:1) calculated by univariate ROC analysis. ROC: Receiver operating characteristic; SVM: Support vector machine; AUC: Area under the curve; MAFLD: Metabolic dysfunction-associated fatty liver disease; SA: Subclinical atherosclerosis; LPC: Lysophosphatidylcholine.

3 讨论

本研究通过非靶向代谢组学鉴定出 MAFLD 患者中发生 SA 的排名前 5 的差异代谢物为[LPC(18:3)、白屈菜红碱、N, N-六亚甲基硫代氨基甲酸-S-乙酯、N-乙基-邻巴豆酰甲基苯胺、LPC(18:1)]。差异代谢物主要富集于组氨酸代谢、鞘脂代谢、甘油磷脂代谢、不饱和脂肪酸的生物合成、色氨酸代谢、牛磺酸与亚牛磺酸代谢等通路。这为 MAFLD 患者 CVD 风险的超早期检测及精准防控提供了一种新的非侵入性诊断模式。

LPC 是卵磷脂代谢的中间产物, 具有广泛的生物学效应, 在动脉粥样硬化的发生和发展过程中发挥重要的作用^[24]。LPC 主要在肝脏代谢, 在肝脏疾病和肝毒性中可发生明显变化^[25]。LPC 是氧化型低密度脂蛋白(oxidized low-density lipoprotein, ox-LDL)中主要的活性成分之一, 是一个导致动脉粥样硬化的前炎症因子。在非氧化型低密度脂蛋白的总磷脂酰胆碱(phosphatidylcholine, PC)中 LPC 只占 1%~5%, 在氧化过程中 40%~50% 的 PC 被转化成 LPC, 后者含量提高 30 倍以上^[26]。本研究发现 MAFLD 患者发生动脉粥样硬化时 LPC(18:3)和 LPC(18:1)均上调, Shao 等^[19]在 120 例 MAFLD 患者及 60 例对照研究中也发现 MAFLD 患者发生颈动脉斑块时 LPC(18:2)和 LPC(20:2)均上调。刘丽娟等^[27]发现在钙化斑块中钙沉积周围 LPC(18:0)含量明显增多, 钙化及钙沉积区域 LPC(18:0)含量变少。由此可见, MAFLD 患者发生 SA 的关键代谢物可能是 LPC, 这可能与 ox-LDL 所致的动脉慢性炎症有关。研究^[24]表明, LPC 通过促进血管内皮细胞的凋亡和黏附因子表达, 促进血管平滑肌细胞的增殖、迁移和炎症细胞趋化因子表达, 诱导血管内皮细胞和巨噬细胞等分泌白细胞介素(interleukin, IL)-1、IL-6、IL-4、肿瘤坏死因子 α (tumor necrosis factor- α , TNF- α)、趋化因子及黏附分子等细胞因子, 导致炎症细胞趋化并浸润到内皮下组织, 从而促进动脉粥样硬化。综上所述, LPC 可能是 MAFLD 发生 SA 的关键标志物, 但仍需在后续大样本的前瞻性队列中验证。

白屈菜红碱是一种来源于罂粟科白屈菜植物的异喹啉类生物碱, 具有抗炎、抗病毒、抑菌、抗肿瘤、抗肝纤维化等多种生物活性, 也是目前应用最广泛的特异性蛋白激酶 C(protein kinase C, PKC)抑制剂。PKC 信号通路的过度激活与血管内皮功能障碍、平滑肌细胞增殖及氧化应激密切相关, 这些都是动脉粥样硬化发生、发展的关键病理环节^[28-31]。因此, 白屈菜红碱可能是一种区分 MAFLD 患者是否发生动

脉粥样硬化的潜在标志物。此外, 本研究首次发现 N, N-六亚甲基硫代氨基甲酸-S-乙酯和 N-乙基-邻巴豆酰甲基苯胺与 MAFLD 患者的 SA 相关, 前者是一种选择性芽前除草剂(俗称禾草敌), 后者为一种有机合成中间体, 主要用于染料合成或特殊高分子材料的制备, 此结果有待进一步扩大样本量或在动物实验中进行验证。本研究发现的 5 种差异代谢物对 MAFLD 患者是否发生 SA 的诊断精确度较高, 可在 MAFLD 中有效区分 SA 发生的风险。但此模型尚未进行外部验证, 仅提供一定的数据参考, 还需要进行大样本的前瞻性队列研究进行验证。

本研究中的差异代谢物主要富集于组氨酸代谢、鞘脂代谢、甘油磷脂代谢、不饱和脂肪酸的生物合成、色氨酸代谢、牛磺酸与亚牛磺酸代谢等通路。郝有丽等^[32]在黄金地鼠模型中发现阿托伐他汀在改善 MAFLD 模型鼠血脂紊乱的同时可引起肝功能损伤, 其机制可能与胆汁酸类、甘油磷脂类和脂肪酸类等内源性代谢物水平紊乱相关(原研究使用 NAFLD 诊断标准)。多项研究^[33-36]证实脂代谢中的甘油磷脂代谢、鞘脂代谢及脂肪酸通路在脂肪肝及动脉粥样硬化疾病中发挥关键作用。然而, 与 MAFLD 相关的心血管疾病的脂代谢标志物尚未被证实; 本研究结果验证了先前的发现, 提出 LPC 作为一种潜在的标志物的可能性。研究^[37-42]已证明多种氨基酸代谢(如支链氨基酸、芳香族氨基酸、鸟氨酸、亮氨酸、赖氨酸、甘氨酸、丙氨酸、丝氨酸、半胱氨酸和谷氨酸等)与胰岛素抵抗、脂代谢、氧化应激及炎症相关, 而这些均是 MAFLD 及动脉粥样硬化共同的病理生理机制。牛磺酸是一种广泛存在于哺乳动物组织中的小分子含硫氨基酸, 结构简单且不参与蛋白质合成^[43]。牛磺酸对脂肪代谢的影响主要表现在促进脂肪分解代谢、抑制脂肪合成、减少炎症反应和氧化应激、提高脂联素水平、与胆汁酸结合等途径, 其在 MAFLD 相关的心血管疾病中的作用还需要进一步研究。

本研究存在一定的局限性: 1)样本量较小, 可能会影响结果的普适性; 2)建立的诊断模型尚未完成外部验证, 可能无法进行普适性的评价; 3)由于疾病进展的复杂性, 本研究中的代谢物在动脉粥样硬化进展过程中是否持续存在尚不能确定; 4)对动脉血管早期病变仅使用多动脉血管彩超及踝臂指数(ankle-brachial index, ABI)评估, 未纳入冠脉钙化积分、血管内皮功能等指标。后续可纳入更多 SA 相关指标以便全面评估 MAFLD 患者的血管情况, 并寻找更可靠的生物标志物。

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