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候选基因多态性和肠道菌群及其交互作用与肥胖儿童NAFLD的关联

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[摘 要] 目的：肥胖儿童非酒精性脂肪性肝病(non-alcoholic fatty liver disease，NAFLD)的患病率逐年上升，其发病机制涉及基因与肠道菌群共同作用，但其交互效应尚不清楚。本研究旨在探讨基因多态性、肠道菌群及其交互作用与肥胖儿童NAFLD的关联，为肥胖儿童NAFLD发病机制研究提供新线索。**方法：**采用病例对照研究，选择2020年1月至2021年9月在湖南省儿童医院就诊的87例肥胖儿童NAFLD患者为病例组，以同期就诊的50例肥胖儿童非NAFLD患者为对照组。利用多重聚合酶链反应和高通量测序检测52个候选基因的多态性，结合16S核糖体DNA(16S ribosomal DNA，16S rDNA)测序分析肠道菌群组成。采用Logistic回归分析候选基因多态性与肥胖儿童NAFLD的关联，并在多重比较时应用Benjamini-Hochberg法进行错误发现率(false discovery rate，FDR)校正(预期FDR=0.05)；运用线性判别分析效应量法(linear discriminant analysis effect size，LEfSe)分析、比较肠道菌群属水平上的相对丰度；运用Logistic回归模型、叉生分析和广义多因子降维法(generalized multifactor dimensionality reduction，GMDR)探究基因-菌群2因素间的交互作用，以及多因素间的高阶交互作用与肥胖儿童NAFLD患病风险的关联。**结果：**调整性别、年龄、体重指数后，*PRKAB1* rs6490265及*IL32* rs28372698与肥胖儿童NAFLD均有显著关联(均P<0.05)，且*IL32* rs28372698在FDR校正后仍具有统计学意义(显性与加性模型均P_{adj}=0.035)，但*PRKAB1* rs6490265在各模型中经FDR校正后的关联均无统计学意义。与对照组相比，埃希氏-志贺氏菌属(*Escherichia-Shigella*)、肠杆菌属(*Enterobacter*)、拟杆菌属(*Bacteroides*)、克雷伯杆菌属(*Klebsiella*)、小杆菌属(*Dialister*)、丹毒丝菌属UCG_003(*Erysipelotrichaceae_UCG_003*)在NAFLD组均明显富集(均P<0.05)；与NAFLD组相比，肠源杆菌属(*Intestinibacter*)、粪球菌属(*Coprococcus*)、雷尔氏菌属(*Ralstonia*)、联合乳杆菌属(*Ligilactobacillus*)、梭状杆菌属(*Fusicatenibacter*)、八叠球菌属(*Sarcina*)、*Eubacterium_eligens*_group、假单胞菌属(*Pseudomonas*)在对照组均明显富集(均P<0.05)。相乘交互作用分析表明，*PRKAB1* rs6490265与*Bacteroides*、*Intestinibacter*，*IL32* rs28372698与*Ligilactobacillus*间的相乘交互作用项均具有统计学意义(均P<0.05)；GMDR高阶交互作用分析显示，*IL32* rs28372698、*Ligilactobacillus*与*Intestinibacter*间的交互作用为最佳模型。**结论：***PRKAB1*、*IL32*的基因多态性与肥胖儿童NAFLD患病风险存在潜在关联。*PRKAB1* rs6490265与*Bacteroides*、*Enterobacter*丰度的拮抗交互效应，以及*IL32* rs28372698与*Ligilactobacillus*丰度的协同交互效应，可能共同影响肥胖儿童的NAFLD患病风险。

[关键词] 肥胖儿童；非酒精性脂肪性肝病；基因多态性；肠道菌群；交互作用

Associations of candidate gene polymorphisms, gut microbiota, and their interactions with NAFLD in obese children

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ABSTRACT

Objective: The prevalence of non-alcoholic fatty liver disease (NAFLD) in obese children has been increasing year by year. Its pathogenesis involves the joint effects of genes and gut microbiota, but their interaction effects remain unclear. This study aims to investigate the associations of gene polymorphisms, gut microbiota, and their interactions with NAFLD in obese children, thereby providing new clues for research on the pathogenesis of NAFLD in this population.

Methods: A case-control study was conducted. A total of 87 obese children with NAFLD who visited Hunan Children's Hospital from January 2020 to September 2021 were selected as a case group, and 50 obese children without NAFLD who visited the hospital during the same period were selected as a control group. Polymorphisms in 52 candidate genes were detected using multiplex polymerase chain reaction and high-throughput sequencing, and the composition of the gut microbiota was analyzed using 16S ribosomal DNA (16S rDNA) sequencing. Logistic regression was used to analyze the associations between candidate gene polymorphisms and NAFLD in obese children, and the Benjamini-Hochberg method was applied for false discovery rate (FDR) correction in multiple comparisons, with an expected FDR of 0.05. Linear discriminant analysis effect size (LEfSe) analysis was used to compare the relative abundance of gut microbiota at the genus level. Logistic regression models, crossover analysis, and generalized multifactor dimensionality reduction (GMDR) were used to explore two-factor gene-microbiota interactions and the associations of higher-order multifactor interactions with the risk of NAFLD in obese children.

Results: After adjustment for sex, age, and body mass index, *PRKAB1* rs6490265 and *IL32* rs28372698 were both significantly associated with NAFLD in obese children (both $P<0.05$). *IL32* rs28372698 remained statistically significant after FDR correction, under both the dominant and additive models ($P_{adj}=0.035$), whereas PRKAB1 rs6490265 showed no significant differences in any model after FDR correction. Compared with the control group, *Escherichia-Shigella*, *Enterobacter*, *Bacteroides*, *Klebsiella*, *Dialister*, and *Erysipelotrichaceae_UCG_003* were significantly enriched in the NAFLD group (all $P<0.05$). Compared with the NAFLD group, *Intestinibacter*, *Coprococcus*, *Ralstonia*, *Ligilactobacillus*, *Fusicatenibacter*, *Sarcina*, *Eubacterium_eligens*_group, and *Pseudomonas* were significantly enriched in the control group (all $P<0.05$). Multiplicative interaction analysis showed that the interaction terms between *PRKAB1* rs6490265 and *Bacteroides*, between *PRKAB1* rs6490265 and *Intestinibacter*, and between *IL32* rs28372698 and *Ligilactobacillus* were statistically significant (all $P<0.05$). GMDR higher-order interaction analysis showed that the interaction among *IL32* rs28372698, *Ligilactobacillus*, and *Intestinibacter* was the best model.

Conclusion: Polymorphisms in *PRKAB1* and *IL32* may be potentially associated with the risk of NAFLD in obese children. The antagonistic interaction effects between *PRKAB1* rs6490265 and the abundance of *Bacteroides* and *Enterobacter*, as well as the synergistic

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interaction effect between *IL32* rs28372698 and the abundance of *Ligilactobacillus*, may jointly influence the risk of NAFLD in obese children.

KEY WORDS　obese children; non-alcoholic fatty liver disease; gene polymorphism; gut microbiota; interaction

非酒精性脂肪性肝病(non-alcoholic fatty liver disease，NAFLD)是指无过量饮酒史而出现肝脂肪变性和脂肪蓄积的临床病理综合征[1-2]。近年来，随着儿童肥胖率的上升，肥胖儿童的NAFLD患病率也在不断上升[3]。荟萃分析[4-5]结果显示，一般人群中体重正常儿童的NAFLD患病率约为2.3%，而肥胖儿童患病率为36.1%，肥胖儿童发生NAFLD的风险为正常体重儿童的13.74倍。中国超重和肥胖儿童的NAFLD患病率高达40.4%。然而，NAFLD的确切发病机制尚不清楚，“多重打击学说”认为其发病受到遗传、环境、肠道微生物群等多种因素的共同作用[6]。其中，遗传易感性被认为是NAFLD发病的基础，且肠道菌群在NAFLD发病中具有重要作用。目前，基因、肠道菌群与NAFLD易感性的研究日渐增多，但二者交互作用与NAFLD易感性的研究鲜有报道。此外，由于遗传因素难以改变，探讨可能与其有交互作用的肠道菌群，从可以改变的肠道菌群入手，对于疾病的预防、治疗至关重要。因此，本研究拟探索候选基因多态性与NAFLD的关联，比较肥胖儿童NAFLD患者和单纯肥胖儿童的肠道菌群在属水平上的组成差异，并分析基因、肠道菌群的相加、相乘及高阶交互作用与NAFLD患病风险的关联，为肥胖儿童NAFLD发病机制研究提供新线索。

1 对象与方法

1.1 伦理声明

本研究已获得中南大学湘雅公共卫生学院伦理委员会批准(审批号：XYGW-2018-04)。所有参与者的监护人在研究实施前均签署知情同意书。

1.2 对象

本研究对象为2020年1月至2021年9月在湖南省儿童医院就诊的肥胖儿童。根据肝脏彩色多普勒超声检查结果，将研究对象分为肥胖儿童NAFLD患者(病例组)和肥胖儿童非NAFLD患者(对照组)。肥胖儿童NAFLD患者均由医院超声医师通过肝脏超声检查，参照《非酒精性脂肪性肝病诊疗指南(2010年修订版)》[7]制订的NAFLD诊断标准进行诊断。

病例组纳入标准：1)年龄为6~18岁且符合2018年发布的《学龄儿童青少年超重与肥胖筛查》[8]中肥胖的诊断标准；2)完成二维超声检查；3)儿童及监护人同意参与本研究且签署知情同意书；4)调查问卷完成情况合格。排除标准：1)存在继发性肥胖；2)存在已知的肝病病因(NAFLD除外)；3)正在服用降脂药物；4)未收集到可用来进行基因测序的血液样本；5)患有严重的认知障碍，无法用文字或语言进行沟通；6)入组前1个月内服用过抗生素类药物或者益生菌类制剂等可能干扰肠道正常微生态的药物或制剂。

对照组纳入标准：1)年龄为6~18岁且满足肥胖儿童的标准；2)儿童及监护人同意参与本研究且签署知情同意书；3)肝脏二维超声影像正常；4)肝功能水平正常；5)调查问卷完成情况合格。排除标准：1)有饮酒史或饮酒折合摄入乙醇量>140 g/周(女性>70 g/周)；2)未完善相关检查；3)正在服用降脂药物；4)患有严重的认知障碍，无法用文字或语言进行沟通；5)入组前1个月内服用过抗生素类药物或者益生菌类制剂等可能干扰肠道正常微生态的药物或制剂。

1.3 血样标本的采集及候选基因测定

所有研究对象均在清晨空腹的状态下，抽取静脉血2 mL，置于乙二胺四乙酸二钾真空抗凝管中，在4 ℃、3 000 r/min条件下，离心15 min，分离血浆和血细胞。使用美国QIAGEN公司生产的DNA提取试剂盒提取白细胞DNA，于−20 ℃下保存备用。候选基因的选取基于已发表文献和本课题组前期研究[9-11]结果，采用多重聚合酶链反应(polymerase chain reaction，PCR)和高通量测序技术完成所有候选基因的检测。

1.4 粪便样本的采集及肠道菌群测定

所有研究对象在采样前1周不食用酸奶及益生菌等微生物制品，采样前3天饮食清淡，采样当天自然排便后，迅速用无菌棉签收集中段且与便盘无接触的新鲜粪便至40 mL无菌粪便采样盒，后立即放入−20 ℃冰箱保存，并在冷藏条件下3 h内运至实验室。最后，所有粪便样品统一在实验室无菌操作台中用灭菌冻存管进行分装，每管约2 g，分成2份装在冻

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存管中，置于−80 ℃超低温冰箱保存备用。其中，1份样品用于检测肠道菌群，1份样品备用。采用Illumina NovaSeq平台进行肠道菌群16S核糖体DNA(16S ribosomal DNA，16S rDNA)测序，对16S V4区域进行PCR扩增，引物序列为5'-CCTAYGGGRBGCASCAG-3'，5'-GGACTACNNGGGTATCTAAT-3'。

1.5 统计学处理

使用SPSS 26.0统计学软件进行数据分析。服从正态分布的定量资料以均数±标准差表示，不服从正态分布的定量资料以中位数(第1四分位数，第3四分位数)表示；若2组资料符合若正态分布和方差齐性要求，则采用独立样本*t*检验分析组间差异，否则使用Wilcoxon秩和检验。计数资料以频数(构成比)表示，采用Pearson卡方检验或Fisher精确检验比较组间的差异。使用Logistic回归模型探索候选基因(分为共显性模型、显性模型、隐性模型、加性模型共4种模型)与肥胖儿童NAFLD患病的关联，计算*OR*值及其95% *CI*来评价关联程度。多因素Logistic回归主要校正了性别、年龄及体重指数(body mass index，BMI)等关键因素。考虑到多重比较可能增加假阳性风险，运用Benjamini-Hochberg法对候选基因的关联分析结果进行错误发现率(false discovery rate，FDR)校正，设定预期FDR水平为0.05。鉴于本研究的探索性性质，将原始*P*<0.05且具有生物学功能意义的位点纳入后续的交互作用分析中。采用线性判别分析效应量法(linear discriminant analysis effect size，LEfSe)比较不同组别的肥胖儿童肠道菌群属水平上的相对丰度。采用PICRUSt功能预测分析，对不同组别菌群在代谢通路上的功能差异进行比较。对有关联的基因及组间差异的肠道菌群进行多因素Logistic回归模型和叉生分析，分别探究2因素间的相乘及相加交互作用。采用广义多因子降维法(generalized multifactor dimensionality reduction，GMDR)分析在相乘及相加交互作用中呈阳性的因素之间是否存在高阶交互作用，及其与肥胖儿童NAFLD之间的关联。将数据集随机分为10个大小相等的子集，依次以其中1份作为测试集、其余9份作为训练集进行模型构建与评估，共循环迭代10次。依据训练集精确度、测试集精确度、符号检验及交叉验证一致性系数判断最佳模型。判定标准为：首先筛选出P<0.05且具有统计学意义的模型；在此基础上，训练集精确度、测试集精确度及交叉验证一致性系数越大的模型越佳。采用多因子降维法(1.0.0)软件绘制交互作用的树状图。

2 结 果

2.1 研究对象基本特征

本研究中，共招募到715例儿童。排除不符合肥胖标准儿童84例，未能收集血液样本119例，未能收集粪便样本165例，入组前1个月内服用过抗生素类药物或者益生菌类制剂等可能干扰肠道正常微生态的药物或制剂210例，最终NAFLD组87例，对照组50例符合本研究的纳入、排除标准被纳入研究。

2组间年龄、性别、体重及BMI的差异均无统计学意义(均*P*>0.05)，仅身高的差异具有统计学意义(*P*<0.05，表1)。

表1 研究对象的基本特征

Table 1 Basic characteristics of the participants

组别	*n*	年龄/岁	性别/[例(%)]		身高/cm	体重/kg	BMI/(kg·m^{-2})
			男	女			
NAFLD组	87	11.00(10.00, 12.00)	67(77.0)	20(23.0)	153.40(143.10, 159.00)	63.10(55.50, 76.50)	28.02(25.72, 31.64)
对照组	50	11.00(9.00, 12.00)	37(74.0)	13(26.0)	149.45(140.48, 154.50)	60.80(55.48, 67.13)	27.72(26.61, 28.72)
总体	137	11.00(10.00, 12.00)	104(75.9)	33(24.1)	152.00(142.85, 156.35)	62.60(55.50, 73.65)	27.82(26.23, 30.39)
χ^2/Z		−1.005	0.157		−2.303	−1.793	−0.085
P		0.315	0.691		0.021	0.073	0.932

不符合正态分布的定量资料以中位数(第1四分位数，第3四分位数)表示，组间比较采用Wilcoxon秩和检验。计数资料以频数(构成比)表示，组间比较采用Pearson卡方检验。NAFLD：非酒精性脂肪性肝病；BMI：体重指数。

2.2 候选基因关联分析

选取与肥胖儿童NAFLD关联的52个候选基因位点，包括7个低频罕见突变候选基因(*CES5A* rs148234688、*RAB11FIP1* rs151011205、*ANXA7* rs3750575、*SCG2* rs16864976、*GANC* rs79004308、*SPINT1* rs116845898、*TTC23L* rs77793555)、7个常见

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变异候选基因(*SCD5* rs3733228、*ECI2* rs2326408、*C6orf201* rs659305、*PRKAG2* rs12703159、*CALML5* rs10904516、*ABCC8* rs1800851、*COX11* rs17209882)、13个炎性通路相关候选基因(*CXCL8* rs2227306、*IL17* rs2275913、*IL1B* rs3136558、*IL32* rs28372698、*IL6* rs1800795、*TNF* rs361525、*IL12* rs3212227、*IL21* rs2055979、*GPR120* rs11187533、*TLR4* rs1927914、*TLR5* rs851139、*FXR* rs35724、*TLR2* rs3804099)、12个胰岛素抵抗通路相关基因(*INS* rs689、*INS* rs3842752、*INS* rs3842748、*INSR* rs1799817、*INSR* rs1052371、*SREBF1* rs8066560、*SREBF1* rs2297508、*NR1H3* rs3758674、*NR1H3* rs7120118、*NR1H3* rs7118396、*RXRA* rs4842194、*RXRA* rs1045570)、13个脂质代谢通路相关候选基因(*LEP* rs7799039、*LEPR* rs1137100、*LEPR* rs1171269、*ADIPOQ* rs2241766、*ADIPOQ* rs1501299、*ADIPOQ* rs1063537、*ADIPOR1* rs3737884、*ADIPOR2* rs11061935、*PRKAA1* rs837101、*PRKAB1* rs6490265、*PPARA* rs1800234、*PPARG* rs1801282、*PPARG* rs3856806)。

采用4种传统模型进行分析，包括共显性模型、显性模型、隐性模型、加性模型，对调整混杂因素(性别、年龄、BMI)前、后，52个候选基因位点与肥胖儿童NAFLD的关联进行了分析。结果显示，调整性别、年龄、BMI后，*PRKAB1* rs6490265(共显性模型，TT vs TC：*OR*=0.35，95% *CI* 0.14~0.90；隐性模型：*OR*=0.38，95% *CI* 0.17~0.89；加性模型：*OR*=0.60，95% *CI* 0.37~0.96)、*IL32* rs28372698(共显性模型，AA vs AT：*OR*=2.48，95% *CI* 1.13~5.42，TT vs AT：*OR*=4.17，95% *CI* 1.08~16.12；显性模型：*OR*=2.77，95% *CI* 1.34~5.76；加性模型：*OR*=2.22，95% *CI* 1.25~3.94)的多态性与肥胖儿童NAFLD患病风险的关联均具有统计学意义(均*P*<0.05)，但经FDR多重检验校正后，仅*IL32* rs28372698位点与NAFLD仍存在显著关联(显性模型：P_{adj}=0.035；加性模型：P_{adj}=0.035；表2)。其余各位点在调整前后均未发现与肥胖儿童NAFLD有关。

表2　候选基因多态性与肥胖儿童NAFLD的关联分析

Table 2　Association analysis of candidate gene polymorphisms with NAFLD in obese children

遗传模型	基因型	*OR*(95% *CI*)	*P*	调整*OR*(95% *CI*)*	*P*	P_{adj}†
PRKAB1 rs6490265						
共显性模型	TC	1.00(Ref.)		1.00(Ref.)		
	CC	0.87(0.35~2.19)	0.766	0.83(0.32~2.13)	0.691	0.691
	TT	0.38(0.16~0.95)	0.039	0.35(0.14~0.90)	0.030	0.056
显性模型	TC	1.00(Ref.)		1.00(Ref.)		
	CC+TT	0.58(0.27~1.26)	0.168	0.55(0.25~1.21)	0.138	0.153
隐性模型	TC+CC	1.00(Ref.)		1.00(Ref.)		
	TT	0.41(0.18~0.92)	0.031	0.38(0.17~0.89)	0.025	0.056
加性模型	—	0.63(0.40~0.99)	0.045	0.60(0.37~0.96)	0.035	0.056
IL32 rs28372698						
共显性模型	AT	1.00(Ref.)		1.00(Ref.)		
	AA	2.45(1.14~5.27)	0.022	2.48(1.13~5.42)	0.023	0.056
	TT	4.39(1.15~16.72)	0.030	4.17(1.08~16.12)	0.039	0.056
显性模型	AT	1.00(Ref.)		1.00(Ref.)		
	AA+TT	2.77(1.35~5.70)	0.006	2.77(1.34~5.76)	0.006	0.035
隐性模型	AT+AA	1.00(Ref.)		1.00(Ref.)		
	TT	3.01(0.82~11.02)	0.097	2.93(0.78~10.99)	0.111	0.139
加性模型	—	2.24(1.27~3.95)	0.005	2.22(1.25~3.94)	0.007	0.035

*调整性别、年龄和BMI；†进行错误发现率校正。

2.3 肠道菌群组成比较

采用LEfSe分析，对不同组别的肥胖儿童肠道菌群属水平上的相对丰度进行比较。结果显示，与对照组相比，埃希氏-志贺氏菌属(*Escherichia-Shigella*)、

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肠杆菌属(*Enterobacter*)、拟杆菌属(*Bacteroides*)、克雷伯杆菌属(*Klebsiella*)、小杆菌属(*Dialister*)、丹毒丝菌属UCG_003(*Erysipelotrichaceae_UCG_003*)在NAFLD组均明显富集(均P<0.05)；与NAFLD组相比，肠源杆菌属(*Intestinibacter*)、粪球菌属(*Coprococcus*)、雷尔氏菌属(*Ralstonia*)、联合乳杆菌属(*Ligilactobacillus*)、梭状杆菌属(*Fusicatenibacter*)、八叠球菌属(*Sarcina*)、*Eubacterium_eligens*_group、假单胞菌属(*Pseudomonas*)在对照组均明显富集(均P<0.05，图1A)。对肠道菌群进行PICRUSt功能预测分析表明，以下预测功能在病例组和对照组间存在显著差异：脂代谢、碳水化合物代谢、氨基酸代谢、复制和修复、能量代谢、翻译和细胞信号转导等功能(均P<0.05，图1B)。

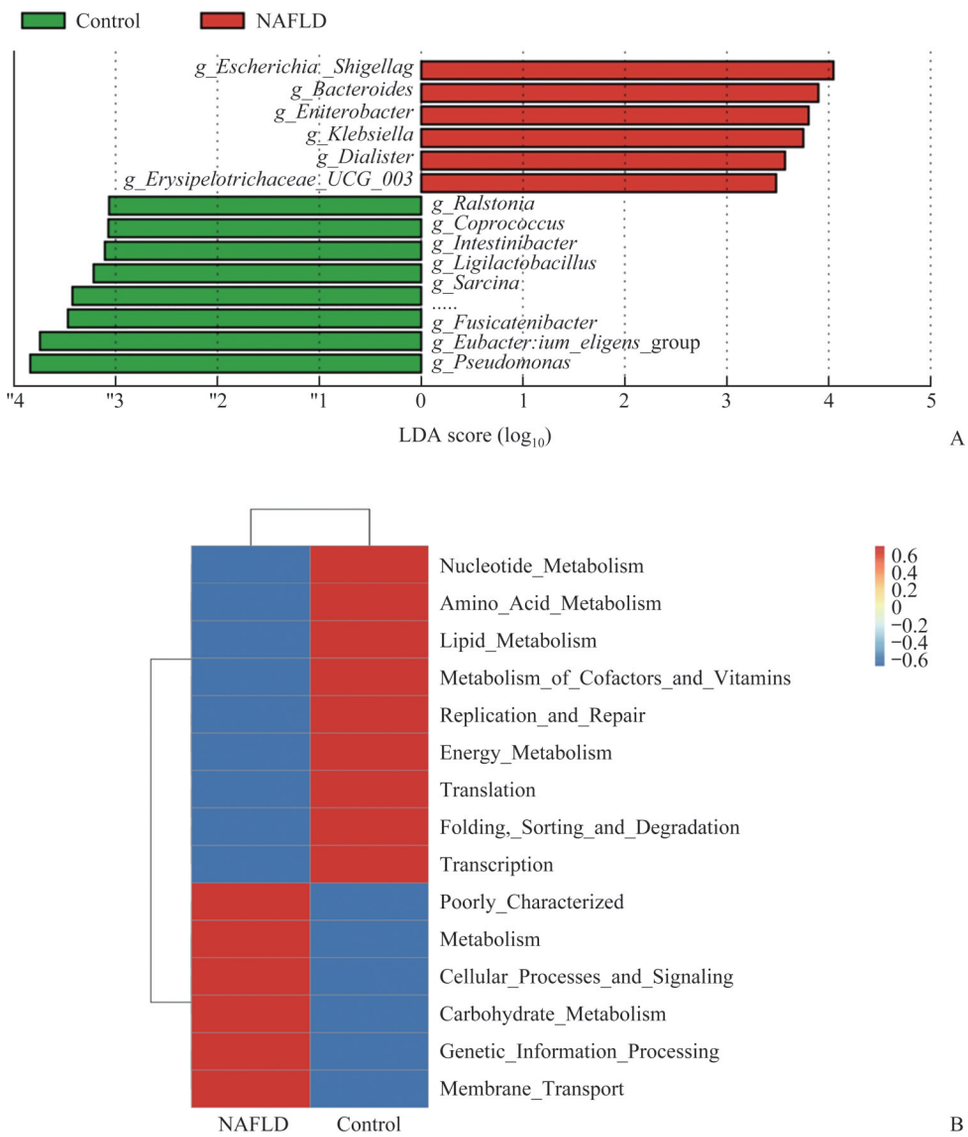


图1 NAFLD组与对照组肠道菌群差异物种及PICRUSt功能预测分析通路

Figure 1 Differential gut microbial genera and PICRUSt-predicted functional analysis pathways between the NAFLD and control groups

A: Bar plot of LDA scores for differentially abundant bacterial genera identified by LEfSe analysis between the NAFLD and control groups. Red bars indicate genera enriched in the NAFLD group, and green bars indicate genera enriched in the control group. Only taxa with a P<0.05 and a LDA score>3 are shown. B: Heatmap of differentially enriched gut microbial functional pathways generated by PICRUSt analysis between the NAFLD and control groups. The color gradient represents the relative abundance of pathways (red for higher abundance, blue for lower abundance). NAFLD: Non-alcoholic fatty liver disease; LDA: Linear discriminant analysis; LEfSe: Linear discriminant analysis effect size.

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2.4 交互作用分析

分别分析了*PRKAB1* rs6490265、*IL32* rs28372698 2个候选基因位点与上述14个肠道菌群的相加、相乘交互作用与肥胖儿童NAFLD的关联。结果显示，*PRKAB1* rs6490265与*Bacteroides*、*Intestinibacter*，*IL32* rs28372698与*Ligilactobacillus*间的相乘交互作用项具有统计学意义(P<0.05)。*PRKAB1* rs6490265与*Bacteroides*、*Intestinibacter*乘法交互项的*OR*均小于1，提示2因素间存在拮抗相乘交互作用，*IL32* rs28372698与*Ligilactobacillus*的乘法交互项的*OR*大于1，提示2因素间存在协同相乘交互作用(表3)。

用GMDR对上述存在相乘交互作用的2个基因与3个肠道菌群进行高阶交互作用分析，结果显示，各维度的最佳模型均具有统计学意义(均P<0.05)，综合各指标，3维模型为最佳模型，纳入因子包括*IL32* rs28372698、*Ligilactobacillus*与*Intestinibacter*，训练集精确度为0.807，测试集精确度为0.757，符号检验P=0.001，交叉验证一致性系数为10/10(表4、图2A)。树状图显示，GMDR交互模型中*IL32* rs28372698与*Ligilactobacillus*的因子连线呈蓝色，表示2者间未表现出协同升高NAFLD患病风险的交互效应(图2B)。

表3　候选基因与肠道菌群相乘交互作用与肥胖儿童NAFLD的关联分析

Table 3　Association analysis of the interaction between candidate genes and gut microbiota and NAFLD in obese children

因素1	因素2	β	P	*OR*(95% *CI*)
PRKAB1 rs6490265	*Bacteroides*	−2.079	0.018	0.13(0.02~0.70)
PRKAB1 rs6490265	*Intestinibacter*	−2.022	0.016	0.13(0.03~0.68)
IL32 rs28372698	*Ligilactobacillus*	2.273	0.023	9.70(1.36~69.19)

表4　2个基因与3个肠道菌群间的交互作用分析

Table 4　Interaction analyses between 2 genes and 3 gut microbiota

模型维度	最优因子组合(最佳模型)	训练集平衡精确度	测试集平衡精确度	P	*CV*
1	*Ligilactobacillus*	0.784	0.766	0.001	10/10
2	*IL32* rs28372698×*Ligilactobacillus*	0.788	0.740	0.001	7/10
3*	*IL32* rs28372698×*Ligilactobacillus*×*Intestinibacter*	0.807	0.757	0.001	10/10
4	*IL32* rs28372698×*PRKAB1* rs6490265×*Ligilactobacillus*×*Intestinibacter*	0.849	0.739	0.001	10/10
5	*IL32* rs28372698×*PRKAB1* rs6490265×*Ligilactobacillus*×*Bacteroides*×*Intestinibacter*	0.880	0.708	0.001	10/10

调整因素为性别、年龄、BMI。*模型维度3为最佳模型。*CV*：交叉验证一致性系数。

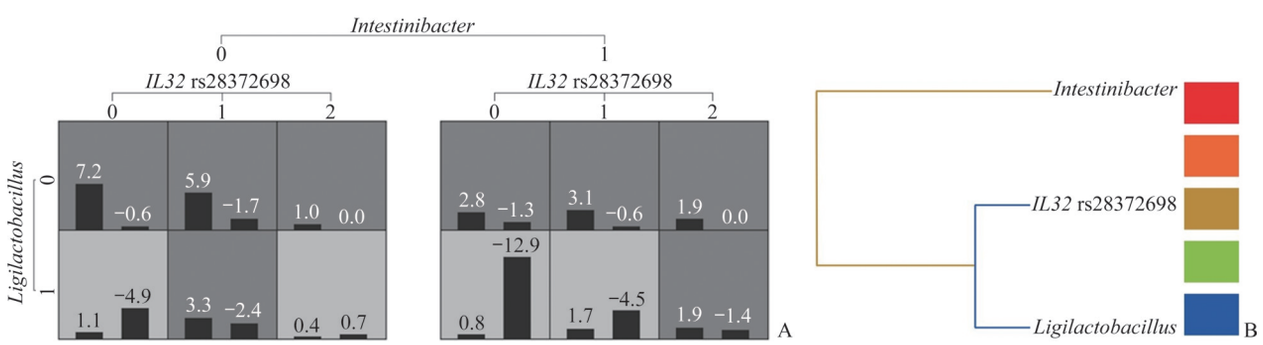


图2　基因与肠道菌群的高阶交互作用分析

Figure 2　Analysis of higher-order interactions between genes and gut microbiota

A: Gene-gut microbiota interaction model of *IL32* rs28372698, *Ligilactobacillus* and *Intestinibacter* in 137 obese children (dark gray grid indicates a high-risk combination, and light gray grid indicates a low-risk combination). The left cylinder in the square indicates the score of the NAFLD group, and the right cylinder indicates the score of the control group. For *Ligilactobacillus* and *Intestinibacter,* 0 indicates a relative abundance less than or equal to the median, and 1 indicates a relative abundance greater than the median. For *IL32* rs28372698, 0 indicates the AT genotype, 1 indicates the AA genotype, and 2 indicates the TT genotype. B: Gene-intestinal flora interaction dendrogram of *IL32* rs28372698, *Ligilactobacillus* and *Intestinibacter* in 137 obese children. The colors range from red representing a synergistic effect, which is the strongest interaction that synergistically increases the risk of NAFLD, to blue representing an irrelevant or redundant correlation between two factors that does not increase the risk of NAFLD.

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3 讨 论

本研究探索了52个候选基因多态性位点与NAFLD易感性的相关性，发现了2个显著相关的基因，分别为*PRKAB1*基因rs6490265位点及*IL32*基因rs28372698位点，其中*IL32*基因rs28372698位点在FDR校正后仍有显著关联；并在属水平上比较了NAFLD组与对照组2组间肠道菌群的差异，得到了14个差异物种，其中*Escherichia-Shigella*、*Enterobacter*、*Bacteroides*、*Klebsiella*、*Dialister*、*Erysipelotrichaceae_UCG_003*在NAFLD组明显富集。

本研究结果初步提示，作为能量代谢调节的关键基因*PRKAB1* rs6490265的多态性在本人群中表现出对肥胖儿童NAFLD的潜在保护性关联。*PRKAB1*基因位于12号染色体长臂(12q24.23)，其编码的蛋白质为AMP活化蛋白激酶(AMP-activated protein kinase，AMPK)的非催化β亚基，该亚基可能是AMPK活性的正调节因子，AMPK活化后可通过磷酸化乙酰辅酶A羧化酶促进脂肪酸氧化、抑制脂质合成关键酶的表达，从而减少肝脂质沉积[12-14]。

本研究还发现*IL32*基因rs28372698多态性与肥胖儿童NAFLD患病风险呈正相关。*IL32*基因位于16号染色体短臂(16p13.3)，是一种蛋白质编码基因。已有研究[15-16]显示，在NAFLD患者中*IL32*表达上调，该基因与NAFLD活动评分、谷草转氨酶、谷丙转氨酶及稳态模型评估的胰岛素抵抗指数相关，用重组*IL32*处理对照人肝细胞会导致胰岛素抵抗。

本研究通过NAFLD组和对照组2组间肠道菌群差异物种的比较，在属水平上得到了14个差异物种，其中*Escherichia-Shigella*、*Enterobacter*、*Bacteroides*、*Klebsiella*、*Dialister*、*Erysipelotrichaceae_UCG_003*在NAFLD组明显富集；与NAFLD组相比，*Intestinibacter*、*Coprococcus*、*Ralstonia*、*Ligilactobacillus*、*Fusicatenibacter*、*Sarcina*、*Eubacterium_eligens*_group、*Pseudomonas*在对照组明显富集。目前已有多项研究[17-20]显示*Escherichia-Shigella*、*Enterobacter*、*Klebsiella*、*Bacteroides*等菌属在NAFLD组中的丰度较对照组显著增加，亦有多项研究[17, 21-23]显示*Intestinibacter*、*Coprococcus*、*Fusicatenibacter*等菌属在NAFLD组中的丰度较对照组显著降低，与本研究结果一致。肠道菌群可通过多种机制导致NAFLD，本研究在病例组与对照组之间发现的存在显著差异的肠道菌群功能主要与脂代谢、碳水化合物代谢、氨基酸代谢、能量代谢、翻译和细胞信号转导等功能相关，能量代谢稳态与NAFLD的关联在以往研究[24-25]中已有报道。此外，肠道菌群亦可通过其他机制导致NAFLD，如在脂代谢和碳水化合物代谢方面，肠道菌群通过增加碳水化合物发酵为短链脂肪酸，随后刺激肝中甘油三酯的从头合成[26-27]；细菌衍生的毒素(如脂多糖)，可激活肝巨噬细胞中的促炎性细胞因子产生，导致肝细胞炎症[28]。肠道菌群也可通过刺激肝Kupffer细胞中的Toll样受体9依赖性促纤维化途径而导致肝纤维化[29]。由此可见，肠道菌群在NAFLD发生、发展中起重要作用。

当前在NAFLD领域，关于基因与生活方式(如饮食、吸烟等)交互作用的研究已经较为深入，但是关于基因与肠道菌群交互作用的研究相对匮乏。本研究利用相乘交互作用分析和GMDR模型对基因和肠道菌群的交互作用进行了分析，为NAFLD的发病机制研究提供了新线索。本研究通过相乘交互作用分析，揭示了*PRKAB1*基因上的rs6490265位点与*Bacteroides*及*Enterobacter*菌群间存在显著的拮抗交互作用，同时发现*IL32*基因上的rs28372698位点与*Ligilactobacillus*菌群间存在协同交互作用。这些发现为理解基因与肠道菌群间的复杂关系提供了新的视角。为了更深入地剖析NAFLD中可能的基因-环境交互作用，本研究采用了高阶分析方法——GMDR模型，该方法有效克服了传统Logistic回归在处理基因-环境相互作用时面临的“维度灾难”问题[30]，从而能够更准确地识别和表征这些复杂的交互关系。通过GMDR模型的分析，本研究发现*IL32* rs28372698、*Ligilactobacillus*及*Enterobacter*之间的交互作用构成了最优组合模型。这一发现不仅进一步证实了基因与肠道菌群在NAFLD发病中的重要作用，也为该病的预防和治疗提供了新的潜在靶点。然而，尽管GMDR模型在处理高维数据方面具有显著优势，但仍存在一定的局限性。例如，随着模型阶数的增加，数据过度拟合的风险也随之增大，导致预测误差的增加。此外，GMDR模型目前尚无法直接估计交互作用的主效应及具体的交互作用程度[31]。因此，在未来的研究中，需要进一步完善和优化GMDR模型，以更全面地揭示基因-环境交互作用的复杂机制。同时，结合其他生物学和临床数据，进行多维度的综合分析，将有助于更深入地理解NAFLD的发病机制，并为该病的精准治疗提供有力支持。

本研究也存在一定局限性。首先，本研究为病例对照研究设计，无法推断菌群差异与NAFLD之间的因果时序关系，结论的因果方向性有待纵向研究阐明。其次，本研究纳入的儿童样本量较小，提示本研究的发现具有初步探索性质，可能存在假阳性风险，且在进行多因素及高阶交互分析时统计效能

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受限，结果的稳定性有待在大样本研究中验证。同时，本研究未通过功能实验验证相关位点的分子机制，且16S rDNA扩增子测序及PICRUSt功能预测的精度有限，相关结论需后续宏基因组及功能实验进一步验证。再次，本研究在进行LEfSe菌群差异分析和PICRUSt功能预测分析时未进行多重假设检验校正，具有一定的假阳性风险。此外，GMDR交互分析中的最佳模型维度3的训练精确度为0.807，测试集精确度为0.757，降幅达6.2%，提示存在中度过度拟合，模型的泛化能力有待独立样本验证。最后，本研究的儿童人群样本仅来源于湖南省内单中心医疗机构，并且在选择对照组时未进行个体匹配，在人群样本的代表性方面存在一定的局限性。

综上，本研究在52个候选基因中发现2个基因位点(*PRKAB1*基因rs6490265位点、*IL32*基因rs28372698位点)与肥胖儿童NAFLD有关；肠道菌群14种菌属与肥胖儿童NAFLD患病有关。同时，基因与肠道菌群存在交互作用，如*PRKAB1* rs6490265与*Bacteroides*、*Enterobacter*的拮抗交互作用，*IL32* rs28372698与*Ligilactobacillus*的协同交互作用，以及*IL32* rs28372698、*Ligilactobacillus*、*Enterobacter*间的高阶交互作用。上述研究成果为NAFLD的发病机制研究开辟了新的方向，也为该病的预防和治疗提供了新的潜在靶点。然而，鉴于NAFLD发病机制的复杂性，未来仍需进一步的研究来验证和拓展这些发现，以期为该病的精准治疗和个体化干预提供更加全面和深入的理论支持。

作者贡献声明：韦彩连　研究设计，数据统计与分析，论文撰写与修改；潘雄峰　研究设计，数据统计与分析；刘耀星　数据统计与分析，论文修订；黄智航、韦佳　样本收集与检测；罗家有、罗米扬　论文设计、指导及修改。所有作者阅读并同意最终的文本。

利益冲突声明：作者声称无任何利益冲突。

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