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脂肪细胞因子通路基因多态性与肥胖儿童 非酒精性脂肪性肝病的关联

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[摘要] 目的: 非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD)存在明显的遗传易感性, 脂肪细胞因子通过参与胰岛素抵抗和肝脏脂肪变性等过程, 在NAFLD的发生和发展中发挥重要作用, 但参与脂肪细胞因子通路的基因与NAFLD之间的关联仍不明确。本研究旨在探索脂肪细胞因子通路的基因多态性位点及其交互作用与肥胖儿童NAFLD的关联。**方法:** 采用病例对照研究, 将肥胖儿童分为NAFLD组和对照组。采集受试者外周静脉血2 mL, 提取DNA后采用多重PCR和高通量测序对脂肪细胞因子通路的14个单核苷酸多态性(single nucleotide polymorphism, SNP)进行分型检测。采用单因素及多因素Logistic回归分析各SNP与肥胖儿童NAFLD的关联。基于显性模型, 联合使用交叉分析和Logistic回归分析相加或相乘交互作用。采用广义多因子降维法(generalized multifactor dimensionality reduction, GMDR)检测14个SNP之间基因-基因交互作用与肥胖儿童NAFLD之间的关联。**结果:** 共纳入1 022例儿童, NAFLD组与对照组各511例。在调整年龄、性别、BMI后, 多因素Logistic回归结果显示: *PPARG* rs1801282在3个遗传模型中与肥胖儿童NAFLD存在关联, 分别是杂合子模型(CG vs CC, $OR=0.58$, 95% CI 0.36~0.95, $P=0.029$)、显性模型(CG+GG vs CC, $OR=0.62$, 95% CI 0.38~1.00, $P=0.049$)、超显性模型(CC+GG vs CG, $OR=1.72$, 95% CI 1.06~2.80, $P=0.028$); *PRKAG2* rs12703159在4个遗传模型中与肥胖儿童NAFLD存在关联, 分别是杂合子模型(CT vs CC, $OR=1.51$, 95% CI 1.10~2.07, $P=0.011$)、显性模型(CT+TT vs CC, $OR=1.50$, 95% CI 1.10~2.03, $P=0.010$)、超显性模型(CC+TT vs CT, $OR=0.67$, 95% CI 0.49~0.92, $P=0.012$)、加性模型(CC vs CT vs TT, $OR=1.40$, 95% CI 1.07~1.83, $P=0.015$)。但*PPARG* rs1801282与*PRKAG2* rs12703159间的相乘及相加交互作用均与肥胖儿童NAFLD不存在关联。经GMDR分析, 调整年龄、性别、BMI后, 14个SNP之间的交互作用均无统计学意义(均 $P>0.05$)。**结论:** *PPARG* rs1801282、*PRKAG2* rs12703159突变型与肥胖儿童NAFLD存在关联, 但未发现SNP交互作用与肥胖儿童NAFLD之间的关联。

[关键词] 肥胖儿童; 非酒精性脂肪性肝病; 脂肪细胞因子; 单核苷酸多态性; 交互作用; 广义多因子降维法

Association of adipocytokine pathway gene polymorphisms with NAFLD in obese children

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ABSTRACT

Objective: Non-alcoholic fatty liver disease (NAFLD) has significant genetic susceptibility. Adipocytokines play a crucial role in NAFLD development by participating in insulin resistance and hepatic steatosis. However, the association between adipocytokine pathway genes and NAFLD remains unclear. This study aims to explore the association of gene polymorphisms in the adipocytokine pathway and their interactions with NAFLD in obese children.

Methods: A case-control study was conducted, dividing obese children into NAFLD and control groups. Peripheral venous blood (2 mL) was collected from each participant for DNA extraction. A total of 14 single nucleotide polymorphisms (SNP) in the adipocytokine pathway were genotyped using multiplex PCR and high-throughput sequencing. Univariate and multivariate Logistic regression analyses were used to assess the association between SNP and NAFLD in obese children. Dominant models were used to analyze additive and multiplicative interactions via crossover analysis and Logistic regression. Generalized multifactor dimensionality reduction (GMDR) was used to detect gene-gene interactions among the 14 SNPs and their association with NAFLD in obese children.

Results: A total of 1 022 children were included, with 511 in the NAFLD group and 511 in the control group. After adjusting for age, gender, and BMI, multivariate Logistic regression showed that *PPARG* rs1801282 was associated with NAFLD in the obese children in 3 genetic models: heterozygote model (CG vs CC, $OR=0.58$, 95% CI 0.36 to 0.95, $P=0.029$), dominant model (GG+CG vs CC, $OR=0.62$, 95% CI 0.38 to 1.00, $P=0.049$), and overdominant model (CC+GG vs CG, $OR=1.72$, 95% CI 1.06 to 2.80, $P=0.028$). *PRKAG2* rs12703159 was associated with NAFLD in 4 genetic models: heterozygous model (CT vs CC, $OR=1.51$, 95% CI 1.10 to 2.07, $P=0.011$), dominant model (CT+TT vs CC, $OR=1.50$, 95% CI 1.10 to 2.03, $P=0.010$), overdominant model (CC+TT vs CT, $OR=0.67$, 95% CI 0.49 to 0.92, $P=0.012$), and additive model (CC vs CT vs TT, $OR=1.40$, 95% CI 1.07 to 1.83, $P=0.015$). No significant multiplicative or additive interaction between *PPARG* rs1801282 and *PRKAG2* rs12703159 was found in association with NAFLD. GMDR analysis, adjusted for age, gender, and BMI, revealed no statistically significant interactions among the 14 SNPs (all $P>0.05$).

Conclusion: Mutations in *PPARG* rs1801282 and *PRKAG2* rs12703159 are associated with NAFLD in obese children. However, no gene-gene interactions among the SNP are found to be associated with NAFLD in obese children.

KEY WORDS

obese children; non-alcoholic fatty liver disease; adipocytokine; single nucleotide polymorphism; interaction; generalized multifactor dimensionality reduction

非酒精性脂肪性肝病(non-alcoholic fatty liver disease, NAFLD)已成为全球成人和儿童最常见的慢性肝病,其发病率仍呈上升趋势^[1]。研究^[2-6]结果显示肥胖儿童NAFLD患病率为20%~50%,已经达到或超

过成年人患病率。NAFLD与代谢综合征、2型糖尿病、动脉硬化性心血管疾病等慢性疾病的发生密切相关^[7-10];此外,在儿童中更容易发生以肝脏纤维化为特征的肝细胞损伤,后果十分严重。

脂肪细胞因子通过介导炎症、免疫及内分泌反应,参与胰岛素抵抗和肝脏脂肪变性等过程,影响肝脂肪沉积^[11]。在种类繁多的脂肪细胞因子中,脂联素和瘦素与NAFLD之间的关联研究较多。其中,脂联素可诱导AMP活化的蛋白质激酶(AMP-activated protein kinase, AMPK)和过氧化物酶体增殖物激活受体 α (peroxisome proliferator activated receptor α , PPAR α)通路的激活,影响脂肪酸的氧化水平,增加胰岛素敏感性,调节脂质代谢,抵抗脂肪变性,在NAFLD的发生和发展中发挥重要作用^[12-13]。

NAFLD作为一种由遗传、环境等多因素综合影响的复杂疾病,既往流行病学研究、家系研究、双生子研究等均证实NAFLD存在明显的遗传易感性^[14-17],如*PNPLA3*、*GCKR*、*APOC3*、*TM6SF2*等均为NAFLD的易感基因,但参与脂肪细胞因子通路的基因与NAFLD之间的关联仍不明确。本研究旨在分析脂肪细胞因子通路相关基因及其单核苷酸多态性(single nucleotide polymorphisms, SNP)位点与肥胖儿童NAFLD的关联,并进一步探索基因-基因间交互作用与肥胖儿童NAFLD的关联。

1 对象与方法

1.1 对象

本研究为病例对照研究,纳入2019年5月至2021年9月于湖南省儿童医院儿童保健所就诊的6~18岁肥胖儿童作为研究对象。由2名临床医生根据NAFLD诊断标准确诊为NAFLD者纳入NAFLD组,未患NAFLD及相关疾病者纳入对照组。

纳入标准:1)本人和/或其监护人充分了解本研究的内容和目的,自愿参与本研究项目并签署纸质知情同意书者;2)6~18岁的中国儿童;3)根据肥胖诊断标准确诊为肥胖者。

排除标准:1)未收集到可用以基因测序的血液样本;2)年龄、性别、体重指数(body mass index, BMI)等基本信息不全者;3)彼此间有亲缘关系者;4)存在继发性肥胖者;5)正在服用降脂药物者。

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1.2 相关诊断标准

1.2.1 肥胖诊断标准

根据2018年发布的《学龄儿童青少年超重与肥胖筛查标准》^[18],首先以各性别、年龄别BMI界值对研究对象是否存在肥胖进行判断,再分别以各性别、年龄别BMI界值的1.0、1.2和1.4倍作为I级、II级、III级肥胖的界定标准^[19-21],将纳入的肥胖儿童进行分级(表1)。

1.2.2 NAFLD诊断标准

根据中华医学会肝病学分会脂肪肝和酒精性肝病学组推荐的《非酒精性脂肪性肝病防治指南(2018年更新版)》中的相关诊断标准^[22],临床医生结合肝脏B超检查结果和相关病史明确所有研究对象的NAFLD诊断。B超结果满足以下3项中任意2项者可视为脂肪肝:1)肝脏前场回声弥漫性增强(出现“明亮肝”),回声强于肾脏;2)肝内管道结构显示不清;3)肝脏远场回声逐渐衰减。同时应满足:1)无饮酒史或过去12个月每周饮酒男性<210 g,女性<140 g;2)未应用乙胺碘呋酮、氨甲蝶呤、他莫昔芬、糖皮质激素等药物;3)未患丙型肝炎病毒感染、肝豆状核变性、自身免疫性肝炎等可导致脂肪肝的特定肝病,并除外全胃肠外营养、 β -脂蛋白缺乏症、乳糜泻等导致脂肪肝的特殊情况。

表1 6~18岁儿童青少年性别年龄别BMI筛查肥胖界值

Table 1 Age-specific BMI threshold screening obesity for children aged 6 to 18 years old

肥胖分级	年龄别BMI界值/(kg·m ⁻²)													
	6~<7岁	7~<8岁	8~<9岁	9~<10岁	10~<11岁	11~<12岁	12~<13岁	13~<14岁	14~<15岁	15~<16岁	16~<17岁	17~<18岁	18~<19岁	
男	I级	17.7	18.7	19.7	20.8	21.9	23.0	24.1	25.2	26.1	26.6	27.1	27.6	28.0
	II级	21.2	22.4	23.6	25.0	26.3	27.6	28.9	30.2	31.3	31.9	32.5	33.1	33.6
	III级	24.8	26.2	27.6	29.1	30.7	32.2	33.7	35.3	36.5	37.2	37.9	38.6	39.2
女	I级	17.5	18.5	19.4	20.4	21.5	22.7	23.9	25.0	25.9	26.6	27.1	27.6	28.0
	II级	21.0	22.2	23.3	24.5	25.8	27.2	28.7	30.0	31.1	31.9	32.5	33.1	33.6
	III级	24.5	25.9	27.2	28.6	30.1	31.8	33.5	35.0	36.3	37.2	37.9	38.6	39.2

BMI: 体重指数。

1.3 问卷调查及体格测量

使用统一的问卷调查表,并由经统一培训后的调查员按照统一流程对研究对象开展问卷调查。调查内容包括研究对象的年龄、性别、民族等一般资料,采用《学生健康检查技术规范》^[23]中规定的标准方法测量身高和体重,并计算BMI。

1.4 候选基因及位点的筛选

结合美国国家生物技术信息中心(National Center for Biotechnology Information, NCBI)的dbSNP数据库(<https://www.ncbi.nlm.nih.gov/snp/>),基于连锁不平衡(linkage disequilibrium, LD)选择信息量最大的tag SNP进行与肥胖儿童NAFLD的关联性分析。

首先,结合京都基因与基因组数据库(Kyoto Encyclopedia of Genes and Genomes, KEGG)通路(map04932)、NCBI的基因数据库(<https://www.ncbi.nlm.nih.gov/gene/>)和文献资料进行NAFLD脂质代谢通路相关待测基因筛选;然后,经在线数据库Genome Variation Server 150的“gene name”模块逐一筛选待测基因的tag SNP。相关参数设定如下:选择国际千

人基因组计划中同属东亚人群且样本量较大的日本东京人群,LD参数 r^2 的阈值设定为0.8,次要等位基因频率(minor allele frequency, MAF)大于5%。基于tag SNP筛选结果,参考以下因素进一步筛选待测SNP:1)已有研究证实或提示与NAFLD有关;2)位于外显子功能区域;3)覆盖许多其他位点。

1.5 基因多态性的检测

基因多态性的总体流程(图1):采集研究对象的外周静脉血2 mL用以检测基因的SNP,将血液样本以3 000 r/min离心15 min,分离血浆及血细胞并将其置于-80 °C超低温冰箱保存;采用基因组DNA小提试剂盒(QIAamp DNA Mini Kit, Qiagen, USA)对外周血白细胞中的DNA进行提取后,运用多重PCR技术和高通量测序技术等方法设计各位点的特异性引物,在单管内进行多重PCR扩增,不同的样本以不同的Barcode引物区分,对扩增子进行高通量测序。通过生物信息学方法在测序结果中区分不同的样本,最终获得每个位点的突变信息,完成SNP分型检测^[24]。



图1 基因分型实验流程

Figure 1 Process of genotyping experiment

1.6 统计学处理

采用Epidata 3.1软件创建数据库,并进行相应的数据核对和逻辑核查。采用双人录入法录入问卷数据并导入SPSS 26.0统计学软件对数据进行统计和分析。计数资料采用例(%)描述,组间比较采用 χ^2 检验;符合正态分布的数据采用均数 \pm 标准差表示,组间比较采用 t 检验;不符合正态分布的数据采用中位数(第1四分位数,第3四分位数)表示,组间比较采用Mann-Whitney U 检验。采用 χ^2 检验对对照组的各位点基因型分布频率进行哈迪-温伯格平衡(Hardy-Weinberg equilibrium, HWE)检验。基于6种遗传模型(共显性模型、显性模型、隐性模型、超显性模型、等位基因模型、加性模型,杂合子模型和纯合子模型属于共显性模型),采用单因素及多因素Logistic回归分析各多态性位点与肥胖儿童NAFLD的关联性。基于显性遗传模型,联合使用交叉分析和Logistic回归分析相加或相乘交互作用;多个SNP间的高阶交

互作用采用广义多因子降维法(generalized multifactor dimensionality reduction, GMDR)通过GMDR软件进行分析。采用双侧检验, $P<0.05$ 为差异有统计学意义。

2 结果

2.1 候选基因及位点

共筛选出与脂肪细胞因子通路相关的基因10个、SNP位点14个(表2)。

2.2 研究对象基本情况

共纳入研究对象1 022例,NAFLD组和对照组各511例。2组在年龄、BMI及肥胖严重程度方面的差异均有统计学意义(均 $P<0.05$),性别和民族构成之间的差异均无统计学意义(均 $P>0.05$,表3)。

表2 脂肪细胞因子通路相关基因及其 SNP 位点信息

Table 2 Information of genes related to adipocytokine pathway and its SNP loci

基因	位点	染色体位置	变异类型	等位基因(野生型/突变型)	MAF
<i>ADIPOQ</i>	rs2241766	3:186853103	同义突变	T/G	0.15
<i>ADIPOQ</i>	rs1501299	3:186853334	内含子突变	G/T	0.30
<i>ADIPOQ</i>	rs1063537	3:186856286	3'-非翻译区突变	C/T	0.14
<i>ADIPOR1</i>	rs3737884	1:202944076	内含子突变	C/T	0.13
<i>ADIPOR2</i>	rs11061935	12:1704608	内含子突变	A/G	0.16
<i>LEP</i>	rs7799039	7:128238730	上游2 kb突变	G/A	0.39
<i>LEPR</i>	rs1171269	1:65531119	内含子突变	G/T	0.34
<i>LEPR</i>	rs1137100	1:65570758	错义突变	A/G	0.31
<i>PPARA</i>	rs1800234	22:46219983	错义突变	T/C	0.02
<i>PPARG</i>	rs1801282	3:12351626	错义突变	C/G	0.07
<i>PPARG</i>	rs3856806	3:12434058	同义突变	C/T	0.12
<i>PRKAA1</i>	rs837101	5:40773597	内含子突变	C/T	0.27
<i>PRKAB1</i>	rs6490265	12:119667902	5'-非翻译区突变	C/T	0.23
<i>PRKAG2</i>	rs12703159	7:151807412	内含子突变	C/T	0.17

SNP: 单核苷酸多态性; MAF: 次要等位基因频率。表中所列为千人基因组计划结果。

表3 NAFLD组和对照组的基本情况比较

Table 3 Comparison of the basic information between the NAFLD group and the control group

组别	n	性别[例(%)]		年龄/岁	民族[例(%)]		BMI/ (kg·m ⁻²)	肥胖严重程度[例(%)]		
		男	女		汉族	少数民族		I级	II级	III级
NAFLD组	511	365(71.4)	146(28.6)	13(11, 15)	480(93.9)	31(6.1)	28.80±3.46	312(61.1)	162(31.7)	37(7.2)
对照组	511	356(69.7)	155(30.3)	12(10, 15)	480(93.9)	31(6.1)	26.94±2.77	430(84.1)	64(12.5)	17(3.3)
$\chi^2/t/Z$		$\chi^2=0.381$		$Z=-2.078$	$\chi^2<0.001$		$t=0.381$	$\chi^2=68.668$		
P		0.537		0.038	1.000		<0.001	<0.001		

*计数资料采用例(%)描述, 组间比较采用 χ^2 检验; BMI符合正态分布, 采用均数±标准差表示, 组间比较采用 t 检验; 年龄不符合正态分布, 采用中位数(第1四分位数, 第3四分位数)表示, 组间比较采用Mann-Whitney U 检验。NAFLD: 非酒精性脂肪性肝病; BMI: 体重指数。

2.3 基因型分布及HWE检验

在NAFLD组和对照组, 14个SNP位点的基因型分型成功率均>90%。对照组各基因型频率的实际值与期望值差异均无统计学意义(HWE检验均 $P>0.05$, 表4), 符合HWE定律, 表明本研究的样本来自遗传平衡群体, 代表性较好。

2.4 多态性位点与肥胖儿童NAFLD的关联

单因素Logistic回归分析以及调整混杂因素(性别、年龄、BMI)后的多因素Logistic回归分析结果(表5)显示: *PPARG* rs1801282在3个遗传模型中与肥胖儿童NAFLD存在关联, 分别是杂合子模型(CG vs

CC, $OR=0.58$, 95% CI 0.36~0.95, $P=0.029$)、显性模型(CG+GG vs CC, $OR=0.62$, 95% CI 0.38~1.00, $P=0.049$)、超显性模型(CC+GG vs CG, $OR=1.72$, 95% CI 1.06~2.80, $P=0.028$); *PRKAG2* rs12703159在4个遗传模型中与肥胖儿童NAFLD存在关联, 分别是杂合子模型(CT vs CC, $OR=1.51$, 95% CI 1.10~2.07, $P=0.011$)、显性模型(CT+TT vs CC, $OR=1.50$, 95% CI 1.10~2.03, $P=0.010$)、超显性模型(CC+TT vs CT, $OR=0.67$, 95% CI 0.49~0.92, $P=0.012$)、加性模型(CC vs CT vs TT, $OR=1.40$, 95% CI 1.07~1.83, $P=0.015$); 其余各SNP均与肥胖儿童NAFLD无关(均 $P>0.05$)。

表4 SNP位点基因型分布及HWE检验

Table 4 Genotype distribution and HWE test results of each SNP loci

基因	位点	组别	测序成功/ [例(%)]	基因型分布*						P†
				AA		AB		BB		
				人数	构成比/%	人数	构成比/%	人数	构成比/%	
ADIPOQ	rs2241766	对照	505(98.8)	274	54.3	196	38.8	35	6.9	0.995
		NAFLD	511(100.0)	284	55.6	191	37.4	36	7.0	
ADIPOQ	rs1501299	对照	508(99.4)	264	52.0	212	41.7	32	6.3	0.218
		NAFLD	510(99.8)	280	54.9	201	39.4	29	5.7	
ADIPOQ	rs1063537	对照	510(99.8)	281	55.1	192	37.6	37	7.3	0.595
		NAFLD	509(99.6)	284	55.8	189	37.1	36	7.1	
ADIPOR1	rs3737884	对照	503(98.4)	300	59.6	168	33.4	35	7.0	0.091
		NAFLD	510(99.8)	295	57.8	185	36.3	30	5.9	
ADIPOR2	rs11061935	对照	486(95.1)	247	50.8	210	43.2	29	6.0	0.071
		NAFLD	511(100.0)	246	48.2	227	44.4	38	7.4	
LEP	rs7799039	对照	477(93.3)	267	56.0	181	37.9	29	6.1	0.819
		NAFLD	507(99.2)	281	55.4	193	38.1	33	6.5	
LEPR	rs1171269	对照	501(98.0)	498	99.4	3	0.6	0	0	0.946
		NAFLD	503(98.4)	498	99.0	4	0.8	1	0.2	
LEPR	rs1137100	对照	503(98.4)	364	72.4	128	25.4	11	2.2	0.949
		NAFLD	511(100.0)	350	68.5	145	28.4	16	3.1	
PPARA	rs1800234	对照	511(100.0)	468	91.6	42	8.2	1	0.2	0.955
		NAFLD	511(100.0)	470	92.0	41	8.0	0	0	
PPARG	rs1801282	对照	511(100.0)	461	90.2	50	9.8	0	0	0.245
		NAFLD	511(100.0)	478	93.5	31	6.1	2	0.4	
PPARG	rs3856806	对照	510(99.8)	294	57.6	186	36.5	30	5.9	0.935
		NAFLD	510(99.8)	288	56.5	190	37.2	32	6.3	
PRKAA1	rs837101	对照	505(98.8)	303	60.0	179	35.4	23	4.6	0.596
		NAFLD	510(99.8)	294	57.6	191	37.5	25	4.9	
PRKAB1	rs6490265	对照	436(85.3)	108	24.8	200	45.9	128	29.3	0.092
		NAFLD	593(96.5)	112	22.7	235	47.7	146	29.6	
PRKAG2	rs12703159	对照	508(99.4)	400	78.7	101	19.9	7	1.4	0.828
		NAFLD	509(99.6)	377	74.0	122	24.0	10	2.0	

*AA为野生纯合子, AB为突变杂合子, BB为突变纯合子; †对照组HWE检验结果。SNP: 单核苷酸多态性; HWE: 哈迪-温伯格平衡; NAFLD: 非酒精性脂肪性肝病。

2.5 交互作用分析

调整性别、年龄、BMI后, *PPARG* rs1801282、*PRKAG2* rs12703159 两位点间相乘交互作用无统计学意义($\beta=0.363$, $P=0.508$, $OR=1.44$, 95% CI 0.49~4.22), 不存在相加交互作用[相对超额危险度(relative excess risk of interaction, RERI)和归因比(attributable proportion, AP)的 $OR(95\% CI)$ 分别为 0.157(-1.241~

1.555) 和 0.133(-1.054~1.321), 均包含 0; 协同指数(synergy index, S)的 95% CI 包含 1]。

将脂肪细胞因子通路相关的 10 个基因的 14 个 SNP 位点全部作为分析因子纳入 GMDR 模型, 同时控制性别、年龄、BMI 等混杂因素, 结果显示: 各维度的最优因子组合, 即各维度的最佳模型均无统计学意义(均 $P>0.05$, 表 6)。

表5 脂肪细胞因子通路相关基因的多态性与肥胖儿童NAFLD的关联性分析

Table 5 Analysis of the association between polymorphism of adipocyte cytokine pathway related genes and NAFLD in obese children

基因位点	模型	单因素 Logistic 回归分析		多因素 Logistic 回归分析*		AIC	BIC
		OR(95% CI)	P	OR(95% CI)	P		
PPARG rs1801282	CC	1(Ref.)		1(Ref.)			
	杂合子†(CG)	0.60(0.38~0.95)	0.031	0.58(0.36~0.95)	0.029	1 317.2	1 341.8
	纯合子†(GG)	—	—	—	—		
	显性	0.64(0.40~1.01)	0.053	0.62(0.38~1.00)	0.049	1 318.5	1 338.2
	隐性	—	—	—	—	1 320.1	1 339.8
	超显	1.68(1.05~2.68)	0.029	1.72(1.06~2.80)	0.028	1 317.5	1 337.2
	加性	0.69(0.44~1.07)	0.099	0.67(0.42~1.06)	0.087	1 319.5	1 339.2
	等位	0.69(0.44~1.07)	0.098				
PRKAG2 rs12703159	CC	1(Ref.)		1(Ref.)			
	杂合子†(CT)	1.40(1.04~1.89)	0.028	1.51(1.10~2.07)	0.011	1 309.3	1 333.9
	纯合子†(TT)	1.69(0.72~3.95)	0.226	1.38(0.55~3.43)	0.491		
	显性	1.43(1.07~1.90)	0.017	1.50(1.10~2.03)	0.010	1 307.3	1 327.0
	隐性	1.57(0.67~3.66)	0.298	1.26(0.51~3.13)	0.619	1 313.8	1 333.5
	超显	0.72(0.54~0.98)	0.035	0.67(0.49~0.92)	0.012	1 307.8	1 327.4
	加性	1.37(1.06~1.77)	0.016	1.40(1.07~1.83)	0.015	1 308.1	1 327.8
	等位	1.27(0.98~1.65)	0.075				

*调整因素为性别、年龄、BMI; †杂合子模型和纯合子模型属于共显性遗传模型。—: 无法计算; NAFLD: 非酒精性脂肪性肝病; AIC: 赤池信息量准则; BIC: 贝叶斯信息准则; BMI: 体重指数。

表6 GMDR分析14个SNP位点交互作用与肥胖儿童NAFLD之间关系的结果

Table 6 GMDR analysis result of the interaction of 14 SNP and NAFLD in obese children

最优因子组合(最佳模型)	训练平衡 准确度	测试平衡 准确度	折迭/ 模拟次数	P	交叉验证 一致性
rs12703159	0.536	0.514	6	0.623	9/10
rs837101 rs12703159	0.552	0.455	0	1.000	2/10
rs1063537 rs3737884 rs7799039	0.581	0.486	5	0.623	3/10
rs2241766 rs11061935 rs3856806 rs6490265	0.613	0.478	2	0.989	2/10
rs11061935 rs7799039 rs3856806 rs837101 rs6490265	0.663	0.508	6	0.377	3/10

调整因素为性别、年龄、BMI。GMDR: 广义多因子降维法; NAFLD: 非酒精性脂肪性肝病; SNP: 单核苷酸多态性; BMI: 体重指数。

3 讨论

基于多重打击学说, 脂质代谢异常等引起的肝脏脂肪聚集被认为是NAFLD发病的始动因素, 而众多脂肪细胞因子可影响肝脏脂肪沉积^[11], 但相关机制仍未阐明。脂肪细胞因子通路相关的遗传易感基因是探索肥胖儿童NAFLD遗传机制的重要突破口。

本研究分析了脂肪细胞因子通路相关的10个基因及其14个SNP位点与肥胖儿童NAFLD的关联, 结果显示: 在调整性别、年龄、BMI后, PPARG rs1801282突变型可降低肥胖儿童罹患NAFLD的风险, PRKAG2 rs12703159突变型可增加肥胖儿童罹患

NAFLD的风险。

PPARG基因位于3号染色体, 编码PPAR家族的一个亚型——PPAR γ , PPAR γ 作为脂肪细胞分化的调节因子, 具有调节脂质代谢、抗炎、改善胰岛素抵抗等作用, 与肥胖、糖尿病、动脉粥样硬化和癌症等多种慢性疾病有关。研究^[25-26]显示: PPARG可通过调控miR-21-5p/分泌型卷曲相关蛋白5(secreted frizzled-related protein 5, SFRP5)通路, 减轻非酒精性脂肪性肝炎(non-alcoholic steatohepatitis, NASH)的肝脏炎症和氧化应激反应。既往已有多项研究探索PPARG rs1801282与成年人群NAFLD发病的关联, 但结论并不一致。2014年发表的荟萃分析^[27]显示:

该位点多态性与亚洲人群NAFLD发病有关,而与欧洲人群NAFLD发病无关。此外,有研究^[28]发现 *PPARG* rs1801282 突变会降低DNA结合亲和力,从而影响转录激活,使得脂肪组织中PPAR γ 活性降低,尤其在高加索人中观察到该位点突变与胰岛素敏感性有关。本次研究对象为肥胖儿童,结果发现 *PPARG* rs1801282 与肥胖儿童NAFLD的发生有关,该结论需开展基因功能研究进一步论证。

AMPK是生物能量代谢调节的关键分子,在糖尿病及其他代谢相关疾病的研究中处于核心地位。本研究首次关注AMPK的相关编码基因与NAFLD的关联,结果发现 *PRAK2* rs12703159 与肥胖儿童NAFLD存在关联,携带突变等位基因T的肥胖儿童相对于未携带该突变等位基因者,NAFLD的发病风险更高。究其原因,*PRKAG2*基因位于7号染色体长臂(7q36.1),是AMPK非催化调节 γ 亚基的编码基因之一。AMPK是一种重要的能量感应酶,通过使调节脂肪酸和胆固醇从头合成的关键酶失活来监测细胞能量状态和功能,从而在维持细胞能量稳态中起关键作用^[29],被认为是治疗肥胖、2型糖尿病和癌症等疾病的潜在关键治疗靶标^[30-31]。

本研究尽可能地选择脂肪细胞因子通路上的关键基因及其多态性位点,开展基因多态性与NAFLD的关联研究,但尚不能完全反映该通路所有相关基因及其位点与NAFLD的关联,未来可扩大范围进行探索。本研究还分析了AMPK和PPAR γ 相关编码基因之间的交互作用,探索这2个基因对于NAFLD的共同效应大于(协同)或小于(拮抗)各自单独效应之和(相加)或之积(相乘),但最终的结果是两者之间不存在交互作用。此外,NAFLD是遗传等内在因素及包括膳食营养、肠道菌群、行为生活方式等环境因素共同作用的结果,其发生机制仍有待进一步探索。

综上,本研究发现脂肪细胞因子通路中的 *PPARG* rs1801282 突变型能够降低肥胖儿童发生NAFLD风险, *PRKAG2* rs12703159 突变型能够增加肥胖儿童发生NAFLD风险。本研究结果为NAFLD遗传易感性的研究提供了新的证据,也为脂肪细胞因子通路相关基因及其位点在NAFLD发生和发展中的作用及其机制探索提供了线索。

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