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mTOR 基因 rs2295079 和 rs2295080 位点多态性与川崎病的关联性

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[摘要] 目的: 检测哺乳动物雷帕霉素蛋白(mammalian target of rapamycin, *mTOR*) 基因 rs2295079(G>C), rs2295080(C>A)位点多态性与川崎病(Kawasaki disease, KD)易感性及冠状动脉损伤(coronary artery lesion, CAL)的关系。方法: 采取病例对照研究方法, 选取100例KD患儿(KD组), 其中出现CAL者20例(KD-CAL组), 未出现CAL者80例(KD-WO组); 100例健康儿童作为对照组。检测2个位点的多态性分布。结果: 在KD组与对照组及KD-CAL组与KD-WO组中, rs2295079(G>C), rs2295080(C>A)位点的等位基因频率与基因型频率分布差异无统计学意义($P>0.05$)。结论: 未发现*mTOR*基因rs2295079(G>C), rs2295080(C>A)位点的多态性与KD易感性及CAL存在关联性。

[关键词] 川崎病; *mTOR*基因; 单核苷酸多态性

Association between single nucleotide polymorphism rs2295079 and rs2295080 in *mTOR* gene and Kawasaki disease

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Abstract **Objective:** To investigate the association of single nucleotide polymorphism (SNP) of loci rs2295079 (G>C) and rs2295080 (C>A) in mammalian target of rapamycin (*mTOR*) gene with the susceptibility of Kawasaki disease (KD) and coronary artery lesion (CAL). **Methods:** A case-control study was performed by recruitment of 100 children with KD (KD group) and 100 healthy children (control group) as controls. In the KD group, there were 20 children with CAL (KD-CAL group) and 80 children without CAL (KD-WO group). Genotypes of these two SNP were detected and analyzed. **Results:** No significant difference was found in the frequency distribution of allele and genotype of SNP of loci rs2295079 (G>C) and rs2295080 (C>A) between the KD group and controls,

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the KD-CAL group and KD-WO group. **Conclusion:** No association is found between SNP of loci rs2295079 (G>C) and rs2295080 (C>A) in *mTOR* gene and the development of KD.

Keywords Kawasaki disease; *mTOR* gene; single nucleotide polymorphism

川崎病(Kawasaki Disease, KD)又称皮肤黏膜淋巴结综合征,是一种以全身性血管炎为主要病变的急性发热出疹性疾病,目前病因未明,具有自限性。近年来KD已成为儿童后天性心脏病的首位病因^[1],因此对于KD发病机制的研究也备受关注。目前认为KD的发病与系统性免疫激活及自身抗体形成、细胞因子和炎性介质诱导炎症反应及遗传学因素等相关^[2]。哺乳动物雷帕霉素靶蛋白(mammalian target of rapamycin, *mTOR*),又称FK506蛋白,是一种进化上高度保守的丝氨酸苏氨酸蛋白激酶^[3]。*mTOR*在调节细胞增殖与分化、能量代谢、细胞周期、血管生成、免疫及炎症反应等生理病理过程中皆发挥重要作用^[4-5]。*mTOR*基因的某些单核苷酸多态性(single nucleotide polymorphism, SNP)位点可能影响*mTOR*的转录活性和表达水平,进而影响蛋白质功能。KD和*mTOR*均与炎症及免疫密切相关,但目前对于二者之间的联系鲜见相关研究报道。本研究通过病例对照的方法,探讨*mTOR*基因启动子区rs2295079(G>C)及rs2295080(C>A)位点多态性与KD及冠状动脉损伤(coronary artery lesion, CAL)的关系。

1 对象与方法

1.1 对象

KD组:选取2012至2015年中南大学湘雅三医院住院治疗的100例KD患儿,均符合KD的诊断标准^[6],患儿均为汉族,其中男63例,女37例,男女比例为1.70:1,年龄为2~114(30.33±22.42)个月,所有KD患儿入院前均未经IVIG和阿司匹林治疗。其中KD组根据是否合并CAL又分为KD合并CAL组(KD-CAL组)与KD无CAL组(KD-WO组)。CAL诊断标准参照北京儿童医院心脏超声研究^[6]:冠状动脉内径正常值≤3岁者<2.5 mm; >3岁且≤9岁者<3 mm; >9岁且≤14岁者<3.5 mm;某段管径为邻近段的1.5倍及以上;管壁明显不规则。本组资料中,KD-CAL组20例,KD-WO组80例。

对照组:选择在中南大学湘雅三医院健康管理中心体检的健康儿童100例作为健康对照组,入选儿童均无感染性疾病、风湿性疾病、心血管疾病及既往KD病史等,均为汉族,其中男63例,

女37例,男女比例为1.70:1,年龄为6个月~14岁(41.71±35.32)个月。KD组与健康对照组之间的性别分布差异无统计学意义($P>0.05$)。本研究获得患儿父母的知情同意,并经中南大学湘雅三医院医学伦理委员会的批准。

1.2 KD 组临床资料

100例KD组临床表现:发热100例(100%)、皮疹70例(70%)、球结合膜充血81例(81%),口腔黏膜改变95例(95%),颈部非化脓性淋巴结肿大68例(68%),手足硬肿62例(62%),指、趾端脱皮64例(64%),肛周脱皮56例(56%)。住院期间及出院2月内所有病例做心脏超声检查3次以上,其中发现CAL者20例。

1.3 方法

1.3.1 基因组 DNA 提取

用含抗凝剂乙二胺四乙酸二钾(EDTA-K2)的一次性采血管取静脉血2 mL,采用美国Promega公司的DNA提取试剂盒,按照说明书的要求提取血液基因组DNA,于-20℃冰箱储存备用。

1.3.2 多态性检测

从PubMed网站GenBank系统中查询*mTOR*基因碱基序列,用Primer Premier 5.0软件设计PCR扩增包含*mTOR*基因rs2295079, rs2295080等位点的特异性引物,为750 bp,上游引物为:5'-AGAAGCCGGGTCCTGAAGCT-3',下游引物为:5'-TCTGAAGTCAAATATCCTAATGGT-3',引物由上海生工生物工程股份有限公司合成。采用PCR技术进行扩增,其反应条件如下:95℃预变性5 min;95℃变性30 s,60℃退火30 s,72℃延伸45 s,共35个循环;72℃终末延伸10 min。PCR反应产物在1.5%琼脂糖凝胶电泳,取出凝胶在紫外灯下照相记录电泳图谱。其后,扩增产物送铂尚生物技术(上海)有限公司进行基因测序。应用Chromas软件分析所获测序结果,并利用NCBI网站在线Blast对所获序列进行对比分析。

1.4 统计学处理

采用Hardy-Weinberg遗传平衡定律检验受试对象的各基因型频率是否符合遗传平衡,检验水准

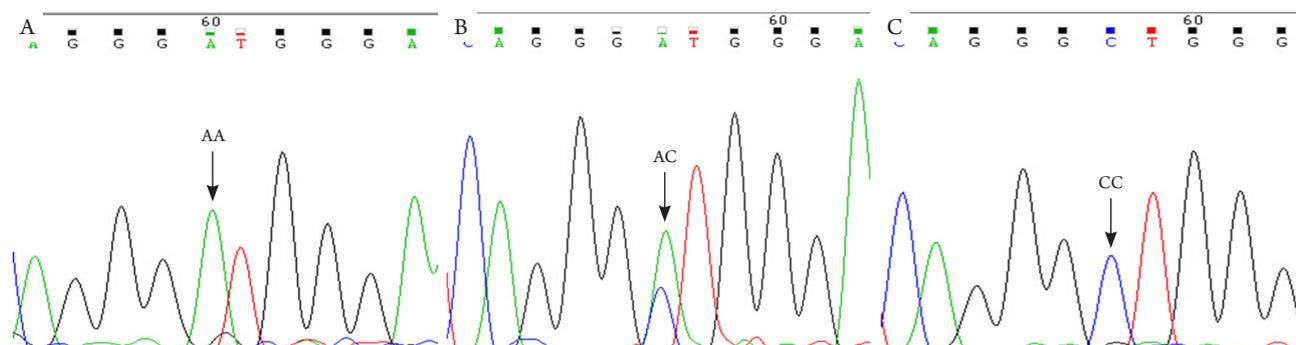


图3 *mTOR*基因rs2295080位点PCR产物测序图

Figure 3 Sequencing of PCR products of *mTOR* gene locus rs2295080 site PCR product

(A)箭头所示A单峰说明该样本为AA纯合子；(B)箭头所示A、C双峰说明该样本为AC杂合子；(C)箭头所示C单峰说明该样本为CC纯合子。

(A) A single peak showed by the arrow indicates AA genotype; (B) A, C double peaks showed by the arrow indicate AC genotype; (C) C single peak showed by the arrow indicates CC genotype.

表1 健康对照组与KD组中*mTOR*基因rs2295079位点多态性的基因型及等位基因频率分布

Table 1 Genotype and allele frequency distribution of *mTOR* gene locus rs2295079 polymorphism in the control group and KD group

组别	n	基因型/[例(%)]			等位基因频率/[例(%)]	
		CC	GC	GG	C	G
健康对照组	100	61 (61.0)	32 (32.0)	7 (7.0)	154 (77.0)	46 (23.0)
KD组	100	66 (66.0)	30(30.0)	4 (4.0)	162 (81.0)	38 (19.0)
χ^2			1.080		0.964	
P			0.583		0.326	

表2 KD-WO组与KD-CAL组中*mTOR*基因rs2295079位点多态性的基因型及等位基因频率分布

Table 2 Genotype and allele frequency distribution of *mTOR* gene locus rs2295079 polymorphism in the KD-WO group and KD-CAL group

组别	n	基因型/[例(%)]			等位基因频率/[例(%)]	
		CC	GC	GG	C	G
KD-WO组	80	55 (68.8)	21 (26.2)	4 (5.0)	131 (81.9)	29 (18.1)
KD-CAL组	20	12 (60.0)	8 (40.0)	0 (0.0)	32 (80.0)	8 (20.0)
χ^2			2.226		0.075	
P			0.329		0.785	

2.5 *mTOR* 基因 rs2295080 位点多态性的基因型分布及等位基因频率比较

KD组与健康对照组比较，*mTOR*基因rs2295080位点的AA，AC，CC基因型分布频率差异无统计学意义($\chi^2=0.367$, $P>0.05$)；KD组与健康对照组的A，C等位基因频率比较，差异

无统计学意义($\chi^2=0.387$, $P>0.05$)。KD-CAL组与KD-WO组的AA，AC，CC基因型分布频率比较，差异无统计学意义($\chi^2=2.719$, $P>0.05$)；KD-CAL组与KD-WO组的A，C等位基因频率比较，差异无统计学意义($\chi^2=0.032$, $P>0.05$ ；表3，4)。

表3 健康对照组与KD组中*mTOR*基因rs2295080位点多态性的基因型及等位基因频率分布Table 3 Genotype and allele frequency distribution of *mTOR* gene locus rs2295080 polymorphism in the control group and KD group

组别	<i>n</i>	基因型/[例(%)]			等位基因频率/[例(%)]	
		CC	GC	GG	C	G
健康对照组	100	63 (63.0)	31 (31.0)	6 (6.0)	157 (78.5)	43 (21.5)
KD组	100	67 (67.0)	28 (28.0)	5 (5.0)	162 (81.0)	38 (19.0)
χ^2			0.367		0.387	
<i>P</i>			0.833		0.534	

表4 KD-WO组与KD-CAL组中*mTOR*基因rs2295080位点多态性的基因型及等位基因频率分布Table 4 Genotype and allele frequency distribution of *mTOR* gene locus rs2295080 polymorphism in the KD-WO group and KD-CAL group

组别	<i>n</i>	基因型/[例(%)]			等位基因频率/[例(%)]	
		CC	GC	GG	C	G
KD-WO组	80	55 (68.8)	20 (25.0)	5 (6.2)	130 (81.3)	30 (18.7)
KD-CAL组	20	12 (60.0)	8 (40.0)	0 (0.0)	32 (80.0)	8 (20.0)
χ^2			2.719		0.032	
<i>P</i>			0.257		0.857	

3 讨论

KD的发病机制尚未明了, 多认为炎症与免疫因素为主要原因。研究^[7]表明: KD在急性期机体免疫被明显激活, T细胞等免疫细胞异常活化, 细胞因子发生级联放大效应, 在细胞因子、细胞间黏附分子及某些炎性介质的介导下, 细胞内酸性物质堆积, 心肌缺血缺氧, 引起急性全身性血管炎, 导致心肌损伤和冠状动脉病变。近年来国内外研究^[8-10]显示: 部分与调节炎症及免疫相关的基因SNP位点多态性与KD的发生密切相关, 如*TNF*基因及IL相关基因等。

*mTOR*基因位于人常染色体1p36.2上, 编码全长2 549个氨基酸的*mTOR*蛋白, 属于磷脂酰肌醇激酶相关蛋白激酶(phosphatidylinositol kinase-related protein kinase, PIKK)家族, 是一种保守的丝氨酸/苏氨酸蛋白激酶。*mTOR*在人体内以2种复合物的形式存在^[11]: 与Raptor蛋白形成复合物, 称为*mTOR*-Raptor复合物(*mTORC1*); 与Rictor蛋白形成的复合物, 称为*mTOR*-Rictor复合物(*mTORC2*)。rs2295079(G>C), rs2295080(C>A)位点位于*mTOR*基因的启动子区域, 二者SNP位点的多态性可能影响该基因的表达, 从而影响*mTOR*基因表达的

信号通路^[12]。研究^[12-15]表明: rs2295079(G>C), rs2295080(C>A)位点多态性引起的*mTOR*基因表达通路的过度活化, 可影响细胞周期的各个环节, 包括细胞的增殖、分化及凋亡, 与多种肿瘤性疾病如胃癌、肾癌、肝癌、结直肠癌及急性白血病的易感性密切相关; 同时可影响细胞的能量代谢, 与2型糖尿病的发病有关^[16]。*mTOR*基因表达信号通路的异常活化参与调节免疫反应各个环节, 包括抗原提呈、巨噬细胞极化、免疫细胞活化、细胞因子应答及细胞迁移, 并通过*mTORC1*及*mTORC2*的选择性活化影响T细胞的增殖和分化^[17-20]。Gao等^[21]发现, *mTOR*基因表达通路的过度活化与冠状动脉疾病患者机体的促炎症状态也有着密切联系。国内外研究中, 使用雷帕霉素(rapamycin)抑制*mTOR*基因表达, 在动物模型中可显著降低病毒性心肌炎的心肌细胞变性^[22]、过敏性哮喘的诱发率^[23]及关节炎的炎症程度^[24], 临床上则用于肾移植术后预防免疫排斥反应的发生^[25], 这也进一步表明*mTOR*基因表达通路的异常活化与炎症及免疫过度激活关系密切。

鉴于KD及*mTOR*与炎症及免疫的诸多联系, *mTOR*基因可能参与KD的发病, 可能机制为*mTOR*基因位点的多态性影响该基因表达,

导致其信号通路异常活化, 继而引起机体免疫反应过度激活, 表现为免疫细胞异常活化, 细胞因子发生级联放大效应, 从而引起急性全身性血管炎, 导致KD的发病。本研究结果显示: 在KD组与对照组及KD-CAL组与KD-WO组中, *mTOR* 基因 rs2295079(G>C), rs2295080(C>A) 位点的等位基因频率和基因型频率分布差异均无统计学意义($P>0.05$), 说明未发现 *mTOR* 基因 rs2295079(G>C), rs2295080(C>A) 位点的多态性与KD易感性及CAL存在关联性。该结果可获如下提示: 1) 研究中样本量不够大, 影响了统计学分析结果的准确性; 2) 可能对于 *mTOR* 基因与KD的关联性, 启动子区的 rs2295079(G>C), rs2295080(C>A) 位点并非责任位点; 3) 可能 *mTOR* 基因在KD发病方面确无重要作用。综上, 关于 *mTOR* 基因多态性与KD的关联性研究仍有待进一步探讨。

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