

· 临床研究 ·

INF- γ /A + 874T 位点基因多态性与 呼吸道合胞病毒毛细支气管炎的相关性

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[摘要] 目的 干扰素- γ (IFN- γ)分泌不足可能在严重呼吸道合胞病毒(RSV)感染的发病中发挥重要作用。IFN- γ /A + 874T 基因位点存在多态性,可能与 RSV 感染的病情存在关联。该研究分析 INF- γ /A + 874T 基因位点在温州地区汉族儿童的分布特征;进一步探讨 INF- γ /A + 874T 位点基因多态性与 RSV 毛细支气管炎易感性、病情的相关性及对鼻咽分泌物 INF- γ 和血清总 IgE 水平的影响。**方法** 采用 DNA 测序法检测 114 例住院的汉族 RSV 毛细支气管炎患儿和 90 例汉族健康体检儿童 INF- γ /A + 874T 位点基因多态性;采用酶联免疫吸附法测定鼻咽分泌物 INF- γ 水平,化学发光法测定血清总 IgE 水平。**结果** 两组均存在 INF- γ /A + 874T 位点基因多态性,以 AA 纯合子基因型为主,其中 RSV 毛细支气管炎组 AA、AT 基因型频率分别为 82.5%,17.5%,对照组分别为 AA 77.8%,AT 21.1%,两组差异无显著性意义;病例组等位基因频率分别为 A 90.4%,T 9.6%,对照组分别为 A 88.3%,T 11.7%,两组差异均无显著性意义。轻度和中重度患儿 INF- γ / + 874 位点 2 种基因型的差异亦无显著性意义。病例组 2 种基因型鼻咽分泌物 INF- γ 及血清总 IgE 水平差异均无显著性意义。**结论** 温州地区汉族儿童存在 INF- γ /A + 874T 位点基因多态性,但未发现该位点变异与 RSV 毛细支气管炎易感性、病情、鼻咽分泌物 INF- γ 及血清总 IgE 水平存在关联。

[中国当代儿科杂志,2009,11(1):21-24]

[关键词] 呼吸道合胞病毒;毛细支气管炎;干扰素- γ ;基因多态性;儿童

[中图分类号] R373.1 **[文献标识码]** A **[文章编号]** 1008-8830(2009)01-0021-04

Association of INF- γ /A + 874T gene polymorphisms with respiratory syncytial virus bronchiolitis

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Abstract: Objective A deficient interferon-gamma (IFN-gamma) response has been involved in the pathogenesis of severe respiratory syncytial virus (RSV) infection. Gene polymorphisms in IFN-gamma/A + 874T have been associated with the susceptibility to asthma and might be related to disease severity of RSV infection. This study investigated the single nucleotide polymorphisms (SNPs) of IFN-gamma/A + 874T in Han children in Wenzhou area and to explore the correlation between gene polymorphisms of IFN-gamma/A + 874T and the susceptibility and disease severity of RSV bronchiolitis, as well as the effect of SNPs upon nasopharyngeal secretions (NPS) IFN-gamma and total serum IgE levels. **Methods** One hundred and fourteen hospitalized children with RSV bronchiolitis and 90 healthy controls were recruited. Sequence analysis was used for detecting the SNPs of IFN-gamma/A + 874T. NPS IFN-gamma levels were measured using ELISA. Total serum IgE levels were assayed using the chemiluminescence method. **Results** IFN-gamma/A + 874T gene polymorphisms were present in both the patient and the control groups. AA and AT genotypes were found in both groups, with a AA frequency of 82.5% vs 77.8% and a AT frequency of 17.5% vs 21.1% ($P > 0.05$). The frequency of allele was 90.4% (A) and 9.6% (T) in the patient group, and 88.3% (A) and 11.7% (T) in the control group, respectively. There were no significant differences in the allele frequency between the two groups. Moreover, no difference was found both in NPS IFN-gamma and total serum IgE levels between AA and AT genotypes in the patient group. There were no significant differences in the variation of IFN-gamma/ + 874 between mild and moderate to severe cases. **Conclusions** IFN-gamma/A + 874T gene polymorphisms were present in Han children in Wenzhou area. Gene variations were not associated with the susceptibility and disease severity of RSV bronchiolitis as well as IFN-gamma and total serum IgE levels.

[Chin J Contemp Pediatr, 2009, 11 (1):21-24]

Key words: Respiratory syncytial virus; Bronchiolitis; Interferon-gamma; Gene Polymorphisms; Child

[收稿日期]2008-06-30;[修回日期]2008-08-22
[基金项目]浙江省医药卫生科技计划(NO:2004B148)。
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呼吸道合胞病毒(RSV)是婴幼儿最常见的下呼吸道感染病原。循证医学证据显示,生命早期的RSV感染是哮喘发生的独立危险因素^[1]。RSV下呼吸道感染至少在病后10年内与气道高反应性(AHR)及哮喘有密切联系^[2]。RSV感染的发病机制迄今尚未完全阐明,干扰素- γ (IFN- γ)分泌不足可能发挥重要作用^[3,4]。IFN- γ 基因位于12号染色体12q24.1,包含4个外显子和3个内含子,其中第一内含子+874位于核转录因子 κ B(NF- κ B)的结合位点中,不同国家和种族人群中普遍存在该位点多态性,影响IFN- γ 与转录因子的结合能力,与IFN- γ 表达及哮喘等疾病的易感性相关^[5,6]。并可能与RSV感染的病情存在关联^[7],但国内尚未见其与RSV感染的相关性报道。因而,我们检测温州地区RSV毛细支气管炎患儿和对照儿童INF- γ /A+874T基因位点,调查多态位点的分布特点,并进一步探讨INF- γ /A+874T位点多态性与RSV毛细支气管炎易感性、病情的相关性以及对鼻咽分泌物(NPS)INF- γ 和血清总IgE水平的影响。

1 材料和方法

1.1 研究对象

1.1.1 对照组 选择在温州医学院附属育英儿童医院健康体检的汉族儿童90例,男70例,女20例。年龄2~24月,平均 8.2 ± 4.0 月。两组均无喘息和特应性疾病史,亦无家族特应性疾病史;性别和年龄的差异均无显著性意义。

1.1.2 病人组 2005年12月至2006年3月首次入住本院呼吸科病房,临床诊断毛细支气管炎,经直接免疫荧光法检测NPS中RSV抗原阳性,确诊为RSV感染的患儿114例,均为汉族,其中男87例,女27例。年龄1~24月,平均 7.04 ± 4.52 月。病情严重程度分型参考Tal等^[8]的评分标准,分为轻度组(≤ 6 分)和中重度组(> 6 分),其中轻度组71例,中重度组43例。除外有双胞胎、早产、先天性心脏病、支气管肺发育不良等基础疾病的患儿。

1.2 方法

1.2.1 DNA测序法进行基因分型 ①采用Chelex-100快速抽提法提取全血基因组DNA模板。②根据INF- γ 序列(NCBI GenBank编号AF375790)合成PCR引物(上海生物工程公司)如下:F:5'-GTT-GCTCACTGGGATT-3'; R:5'-CCTTCCTGTAGGGTATT-3'; PCR扩增条件为:总体积20 μ L,其中10 $\times$ Buffer 2 μ L,即基因组DNA 100 ng, Mg^{2+} 2.5 mmol/L 2 μ L,

NTP Mixture(2.5 mM)2 μ L,引物P1和P2(10 μ M)各1 μ L, Taq 酶 2 U,用灭菌双蒸水补充体积至20 μ L。将反应物置94 $^{\circ}$ C预变性3 min,94 $^{\circ}$ C 30 s,55 $^{\circ}$ C 30 s,72 $^{\circ}$ C 1 min,共30个循环,最后72 $^{\circ}$ C延伸5 min。③纯化(SAP反应):50~80 ng的PCR产物加入0.05 μ L虾碱酶(初浓度7.633 U/mL)和外切酶0.125 μ L(初浓度20 U/mL)后,补去离子水5.825 μ L,予PCR仪中反应37 $^{\circ}$ C 60 min,80 $^{\circ}$ C 15 min后-20 $^{\circ}$ C冰箱保存;④3130x型基因分析仪基因片段测序(美国ABI公司)。

1.2.2 鼻咽分泌物INF- γ 检测 采用酶联免疫吸附法(ELISA)。

1.2.3 血清总IgE检测 采用化学发光法。

1.3 统计学分析

用频率计数法计算各组多态位点基因型及等位基因频率;两组基因型与等位基因比较及基因型Hardy-Weinburg平衡吻合度用卡方(χ^2)检验;等位基因及基因型频率比较用 χ^2 检验;危险因素分析采用logistic回归分析;因方差不齐,两组间总IgE值经对数转换后采用 t 检验;非正态分布且方差不齐数据,结果以中位数表示,采用Mann-Whitney U秩和检验; $P < 0.05$ 认为有显著性意义。以上统计学分析使用SPSS 11.0软件分析。

2 结果

2.1 INF- γ /A+874T基因多态位点的分布

病人组和对照组INF- γ /A+874T位点基因多态性均见AA,AT 2种基因型,以AA基因型为主,对照组还可见TT基因型1例。经检验符合Hardy-Weinburg平衡吻合度($\chi^2 = 1.05, 0.05, P > 0.05$)。其中病人组AA、AT基因型频率分别为82.5%,17.5%,对照组分别为AA 77.8%,AT 21.1%,两组差异无显著性意义($\chi^2 = 0.47, P > 0.05$);病人组等位基因频率分别为A 90.4%,T 9.6%,对照组分别为A 88.3%,T 11.7%,两组差异亦无显著性意义($\chi^2 = 0.43, P > 0.05$; $OR = 1.24, 95\% CI 0.66 \sim 2.32, P > 0.05$), (表1)。INF- γ /A+874T位点DNA测序图见图1~3。

2.2 INF- γ /A+874T基因多态性对NPS INF- γ 和血清总IgE的影响

病人组INF- γ /+874位点AA和AT基因型NPS INF- γ 含量中位数分别为11.89 pg/mL和8.54 pg/mL,两者差异无显著性意义($Z = 1.72, P > 0.05$);血清总IgE对数均值分别为 $1.14 \pm 0.67 IU/mL$ 和

管炎易感性、病情、NPS IFN- γ 和血清总 IgE 水平无关联。Chen 等^[18] 与 Snyder 等^[19] 分别发现特发性血小板减少性紫癜及移植后闭塞性细支气管炎发病与 IFN- γ /+874 位点基因多态性无关,与本研究结果相一致。

IFN- γ 基因多态性与 RSV 感染的相关性研究目前鲜见报道。Gentile 等^[7] 的研究显示 IFN- γ /+874 位点基因多态性与美国儿童 RSV 下呼吸道感染的病情、住 ICU 的时间相关。本研究结果与上述报道不一致的原因除考虑与种族差异有关外,还可能与病人来源和样本量不同有关,该学者的研究对象为 77 例 6 个月以下的 RSV 感染患儿,且有近 20% 入住 ICU;此外,实验方法可能对结果也有影响,该学者未采用 DNA 测序法检测。

[参 考 文 献]

[1] Pérez-Yarza EG, Moreno A, Lázaro P, Mejías A, Ramilo O. The association between respiratory syncytial virus infection and the development of childhood asthma; a systematic review of the literature[J]. *Pediatr Infect Dis J*, 2007, 26 (8):733-739.

[2] Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13[J]. *Am J Respir Crit Care Med*, 2005, 171(1): 137-141.

[3] Schauer U, Hoffjan S, Thoeft T, Bartz H, König S, Fuchs E, et al. Severe respiratory syncytial virus infections and reduced interferon-gamma generation in vitro[J]. *Clin Exp Immunol*, 2004, 138(1):102-109.

[4] Legg JP, Hussain IR, Warner JA, Johnston SL, Warner JO. Type1 and type 2 cytokine imbalance in acute respiratory syncytial virus bronchiolitis[J]. *Am J Respir Crit Care Med*, 2003, 168(6): 633-639.

[5] Tangwattanachuleeporn M, Sodsai P, Avihingsanon Y, Wongpi-abovorn J, Wongchinsri J, Hirankarn N. Association of interferon-gamma gene polymorphism (+874A) with arthritis manifestation in SLE[J]. *Clin Rheumatol*, 2007, 26(11): 1921-1924.

[6] 李志方, 卢健红, 李景柔, 孙筱放, 廖宝平. IFN- γ 基因多态性与小儿哮喘的相关性[J]. *中国优生与遗传杂志*, 2006, 14 (9):22-23.

[7] Gentile DA, Doyle WJ, Zeevi A, Howe-Adams J, Kapadia S, Trecki J, et al. Cytokine gene polymorphisms moderate illness se-

verity in infants with respiratory syncytial virus infection[J]. *Hum Immunol*, 2003, 64(3): 338-344.

[8] Tal A, Bavilski C, Yohai D, Bearman JE, Gorodisher R, Moses SW, et al. Dexamethasone and salbutamol in the treatment of acute wheezing in infants[J]. *Pediatrics*, 1983, 71(1):13-18.

[9] Aberle JH, Aberle SW, Rebhandl W, Pracher E, Kundi M, Popow-Kraupp T. Decreased interferon-gamma response in respiratory syncytial virus compared to other respiratory viral infections in infants[J]. *Clin Exp Immunol*, 2004, 137(1): 146-150.

[10] Lee FE, Walsh EE, Falsey AR, Lumb ME, Okam NV, Liu N, et al. Human infant respiratory syncytial virus (RSV)-specific type 1 and 2 cytokine responses ex vivo during primary RSV infection [J]. *J Infect Dis*, 2007, 195(12): 1779-1788.

[11] Semple MG, Dankert HM, Ebrahimi B, Correia JB, Booth JA, Stewart JP, et al. Severe respiratory syncytial virus bronchiolitis in infants is associated with reduced airway interferon gamma and substance P[J]. *PLoS ONE*, 2007, 2 (10): e1038.

[12] Lee YM, Miyahara N, Takeda K, Prpich J, Oh A, Balhorn A, et al. IFN-gamma production during initial infection determines the outcome of reinfection with respiratory syncytial virus[J]. *Am J Respir Crit Care Med*, 2008, 177(2): 208-218.

[13] 李兰,王智斌,李敏,张剑波,陈昌辉,李波,等. 呼吸道合胞病毒毛细支气管炎患儿 T 细胞亚群检测的临床价值[J]. *中国当代儿科杂志*, 2005, 7(5):421-422.

[14] 张宝琴,宋晓红,宋红霞,罗树舫,雷春莲. 干扰素、白介素 4 在小儿肺炎支原体肺炎合胞病毒性肺炎发病中的作用[J]. *中国当代儿科杂志*, 2002, 4(2):143-144.

[15] 张艳敏,雷春莲,成革胜,杨玉琼. RSV 感染患儿外周血 CD4、CD5、RO⁺、CD45 RA⁺ 表达变化的研究[J]. *中国当代儿科杂志*, 2001, 3(3):260-261.

[16] 朱启镕,俞慧,顾绍庆,王建设,葛艳玲,谢新宝. 干扰素 γ 和肿瘤坏死因子 α 基因单核苷酸多态性与宫内乙型肝炎病毒感染易感性研究[J]. *临床儿科杂志*, 2005, 23(7):446-449.

[17] 张平安,吴健民,李艳. 干扰素 γ 基因内含子 1 +874 位点多态性与乙型肝炎病毒感染关系的研究[J]. *中华流行病学杂志*, 2006, 27(1):41-43.

[18] Chen X, Xu J, Chen Z, Feng X, Zhou Y, Ren Q, et al. Interferon-gamma +874A/T and interleukin-4 intron3 VNTR gene polymorphisms in Chinese patients with idiopathic thrombocytopenic purpura[J]. *Eur J Haematol*, 2007, 79(3):191-197.

[19] Snyder LD, Hartwig MG, Ganous T, Davis RD, Herczyk WF, Reinsmoen NL, et al. Cytokine gene polymorphisms are not associated with bronchiolitis obliterans syndrome or survival after lung transplant [J]. *J Heart Lung Transplant*, 2006, 25 (11):1330-1335.

(本文编辑:吉耕中)