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English translation

论著·临床研究

*MyD88*和*TICAM1*基因多态性及其交互作用与儿童社区获得性肺炎的关联研究

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［摘要］**目的**　探讨髓样分化因子88（myeloid differentiation factor 88，*MyD88*）和Toll样受体衔接分子1（Toll-like receptor adaptor molecule 1，*TICAM1*）基因的单核苷酸多态性及其交互作用与儿童社区获得性肺炎（community-acquired pneumonia，CAP）的相关性。**方法**　前瞻性采用改良多重高温连接酶检测反应技术对2015年8月—2017年9月在延安大学医学院第二附属医院儿科就诊的375例CAP患儿和306例健康体检儿童的*MyD88*和*TICAM1*基因的9个标签位点进行分型，并采用logistic回归分析评价各位点基因型及其交互作用与儿童CAP的关联。**结果**　*TICAM1*基因rs11466711T/C位点多态性与儿童CAP易感性密切相关（*P*<0.05）；rs35747610G/A位点AA基因型可显著降低CAP患儿并发脓毒症的风险（*P*<0.05）；rs6510826G/A位点AA基因型与CAP患儿急性期C反应蛋白水平的增高显著关联（*P*<0.05）。*MyD88*基因rs7744A/G位点GG基因型能显著增加患儿发生呼吸衰竭和循环衰竭的风险（均*P*<0.05）。*MyD88*基因rs7744A/G位点与*TICAM1*基因的rs11466711T/C、rs2292151G/A、rs35299700C/T和rs35747610G/A位点之间存在多个与儿童CAP易感性、严重程度及并发脓毒症风险显著关联的相乘交互作用模式（均*P*<0.05）。**结论**　*MyD88*和*TICAM1*基因多态性及其交互作用与儿童CAP密切相关，对儿童CAP的发生及病情发展具有协同作用。　［中国当代儿科杂志，2023，25（8）：791-799］

［关键词］社区获得性肺炎；*MyD88*基因；*TICAM1*基因；基因多态性；儿童

Association of gene polymorphisms of *MyD88* and *TICAM1* and their interactions with community-acquired pneumonia in children

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Abstract: Objective　To investigate the association of single nucleotide polymorphisms (SNPs) of myeloid differentiation factor 88 (*MyD88*) and Toll-like receptor adaptor molecule 1 (*TICAM1*) and their interactions with community-acquired pneumonia (CAP) in children. **Methods**　Improved multiple ligase detection reaction assay was used for detecting the polymorphisms of nine tagging SNPs of the *MyD88* and *TICAM1* genes in 375 children with CAP who attended the Department of Pediatrics of the Second Affiliated Hospital of Yan'an University Medical School from August 2015 to September 2017 and 306 healthy children who underwent physical examination. A logistic regression analysis was used to evaluate the association between the distribution of genotypes and their interactions with CAP in children. **Results**　The polymorphism of the *TICAM1* gene at rs11466711T/C locus was closely associated with the susceptibility to CAP in children (*P*<0.05). The AA genotype of rs35747610G/A locus significantly reduced risk of sepsis in children with CAP (*P*<0.05). The AA genotype of rs6510826G/A locus was significantly associated with the increase in C-reactive protein level in children with CAP (*P*<0.05). The GG genotype of the *MyD88* gene at rs7744A/G locus significantly increased the risk of respiratory failure and circulatory failure (*P*<0.05). The multiplicative interactions

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between *MyD88* gene rs7744A/G and *TICAM1* gene rs11466711T/C, rs2292151G/A, rs35299700C/T, and rs35747610G/A loci were significantly associated with the susceptibility to CAP, the severity of CAP, and the risk of sepsis in children (P<0.05). **Conclusions** The gene polymorphisms of *MyD88* and *TICAM1* and their interactions are closely associated with CAP in children, with a synergistic effect on the development and progression of CAP in children.

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Key words: Community-acquired pneumonia; *MyD88*; *TICAM1*; Gene polymorphism; Child

儿童社区获得性肺炎（community-acquired pneumonia，CAP）是威胁儿童健康的常见感染性疾病，分子流行病学研究证实，CAP是由遗传因素和环境因素共同作用导致的获得性疾病[1]，相关肺损伤的确切机制十分复杂，且涉及大量受遗传因子影响的细胞和分子免疫过程[2-3]。Toll样受体（Toll-like receptors，TLRs）信号通路在炎症免疫调节中发挥重要作用，TLRs信号通路分为两个途径：一个是髓样分化因子88（myeloid differentiation factor 88，MyD88）依赖途径，另一个则是Toll样受体衔接分子1（Toll-like receptor adaptor molecule 1，TICAM1）依赖途径，分别在细菌脂多糖介导的核因子-κB活化的早期和晚期发挥重要作用，是炎症反应激活和放大过程的重要途径，并对病原体感染的结局起着关键作用[4]。已有研究表明，TLRs信号转导通路相关分子单核苷酸基因多态性（single nucleotide polymorphisms，SNPs）与肺炎易感性密切相关[5-6]，但*MyD88*和*TICAM1*基因多态性与儿童CAP的相关性，以及其介导的相关免疫途径在CAP抗感染免疫过程中的作用尚不明确。为此，本研究选取了TLRs信号通路中分别介导MyD88依赖途径和TICAM1依赖途径的关键衔接分子基因*MyD88*和*TICAM1*的9个标签SNP（tagging SNPs，TagSNPs）作为研究位点，探讨上述基因的多态性及其交互作用与儿童CAP遗传易感性及临床特征的相关性，以期阐述上述基因及其介导的免疫途径在儿童CAP炎性损伤过程中的作用，同时也为儿童CAP的临床治疗提供新的策略及潜在的靶点。

1 资料与方法

1.1 研究对象

前瞻性选择2015年8月—2017年9月在延安大学医学院第二附属医院儿科确诊CAP的375例住院患儿为研究对象，其中男228例，女147例，入院时年龄>28 d且≤15岁，平均年龄（38±22）个月。所有患儿均符合中华医学会儿科学分会呼吸学组修订的儿童社区获得性肺炎管理指南[7]中的诊断标准，并依据该指南中重症肺炎的分级标准，将375例CAP组患儿分为重度CAP亚组（233例）和轻度CAP亚组（142例）。依据儿童脓毒症国际诊疗指南[8]中儿童脓毒症的诊断及分级标准，将375例CAP组患儿分为非脓毒症亚组（175例）、轻度脓毒症亚组（163例）和重度脓毒症亚组（37例，其中包含脓毒症休克患儿）。375例CAP患儿中并发呼吸衰竭32例，并发循环衰竭17例，呼吸衰竭的诊断标准采用2013体外生命支持组织发布的儿童呼吸衰竭指南相关标准[8]，循环衰竭诊断参考儿童脓毒症国际诊疗指南[9]中脓毒症休克的相关标准。CAP组排除标准：（1）自身免疫性疾病或者获得性免疫缺陷病者；（2）肿瘤者；（3）哮喘者；（4）先天性心肺疾病者；（5）遗传性、代谢性疾病者；（6）合并脑性瘫痪、中枢协调障碍者；（7）医院获得性重症肺炎者。对照组为同期于儿童保健门诊健康体检的306例儿童，其中男183例，女123例，平均年龄（38±24）个月。所有对照组儿童血液标本送检前均无肺炎及重症感染性疾病史，排除标准参考CAP组。

两组均为无血缘关系的陕西北部地区汉族人群，且在性别（χ^2=0.069，P=0.792）和年龄（t=0.402，P=0.688）方面比较差异无统计学意义。所有CAP患儿及对照健康儿童入组均得到家长同意，并签署知情同意书。本研究项目得到延安大学医学院第二附属医院伦理委员会的批准（YLYY201505081）。

1.2 候选基因TagSNPs的选取

利用NCBI-SNP（http://www.ncbi.nlm.nih.gov/gen）和1000 Genomes（http://browser.1000genomes.org/Homo_sapiens/UserData/Haploview）数据库下载中国南方汉族人群和中国北京汉族人群候选基因的位置信息（GRCh37版本），再利用Haploview 4.2软件选取TagSNPs位点。入选标准是位置信息于启动子上游到基因末尾前后各扩大2 kb，连锁不平衡参数D'=1，连锁不平衡系数r^2≥0.8且最小等位基因频率≥0.1，具有代表性的TagSNPs位点，最终*MyD88*和*TICAM1*基因共入选9个TagSNPs进行研

究，各位点信息见表1。本研究SNPs分型成功率为99.9%~100%，对照组中9个TagSNPs的基因型频率分布均符合Hardy-Weinberg平衡（均P>0.05），纳入人群的遗传性能稳定，具有群体代表性，见表1。

表1　各TagSNPs信息、功能预测及对照组Hardy-Weinberg平衡分析

基因/位点	位置区域	预测功能	对照组MAF	基因型频数(11/01/00)	对照组HWE(P值)
TICAM1					
rs2292151G/A	第2外显子	p.=(Asp557Asp)	0.471	71/146/89	0.491
rs7255265C/T	第2外显子	p.=(Thr4Thr)	0.366	42/140/124	0.806
rs61231668T/C	第1内含子	–	0.471	70/148/88	0.646
rs35299700C/T	第1内含子	–	0.108	4/58/244	0.765
rs6510826G/A	第1内含子	–	0.446	64/145/97	0.488
rs11466711T/C	第1内含子	–	0.389	51/136/119	0.279
rs10422141A/T	5'上游基因间区	dist=1 656 bp	0.250	19/115/172	1.000
rs35747610G/A	5'上游基因间区	dist=1 867 bp	0.170	7/90/209	0.547
MyD88					
rs7744A/G	3'非编码区	–	0.364	37/149/120	0.459

注：［MAF］最小等位基因频率；［HWE］Hardy-Weinberg平衡值；［11/01/00］纯合低频等位基因/杂合/纯合高频等位基因；［dist］距*TICAM1*基因5' UTR的距离。

1.3　血液标本收集及DNA提取

所有患儿入院当天抽取静脉血2 mL，乙二胺四乙酸抗凝，–20℃暂存备用。采用美国Omega公司全血DNA提取试剂盒，提取抗凝全血基因组DNA。采用1%琼脂糖电泳对所获得的DNA样本进行质量检查及浓度检测，然后根据检测的浓度将样本稀释到工作浓度10 ng/μL后置于–20℃冰箱冻存。

1.4　基因多态性检测

采用上海天昊生物科技公司开发的改良多重高温连接酶检测反应技术对681个样本共9个TagSNPs位点进行基因分型。根据GenBank提供基因序列，运用Primer 5.0软件对位点引物进行设计，各位点引物由上海生工生物有限公司设计合成。本研究实验所有标本的DNA提取、DNA的聚合酶链反应扩增、连接反应检测及原始数据分析均由上海天昊生物科技公司完成，采用ABI 3730XL测序仪（美国ABI公司）测序，采用GeneMapper 4.1软件（美国ABI公司）判读基因型。

1.5　统计学分析

采用plink分析软件完成对照组的Hardy-Weinberg平衡分析。采用SPSS 20.0软件进行统计学分析，P<0.05表示差异有统计学意义。符合正态分布的计量资料用均数±标准差（$\bar{x} \pm s$）表示，组间比较采用两样本t检验；计数资料用例数表示，组间比较采用χ^2检验。采用logistic回归分析基因型与CAP的关联性，并校正性别、年龄及居住环境等显著混杂因素的影响，分析的遗传模型有显性模型、隐性模型、加性模型和共显性纯合/杂合模型。基因间交互作用采用logistic回归模型进行分析，交互作用的统计模型采用相乘模型，等位基因的模型为加性模型。以基因型作为变量，根据风险等位基因个数对其进行赋值，将两基因SNPs的交互项纳入logistic回归模型并对其回归系数进行假设检验，使用Rothman相乘交互作用评价公式判定交互结果，即logistic回归乘积项95%CI不包含1，表示有相乘交互作用，$OR_{A\times B}$=1为无交互作用；$OR_{A\times B}$>1为有正交互作用；$OR_{A\times B}$<1为有负交互作用。

2　结果

2.1　CAP患儿的一般资料比较

重度CAP亚组和轻度CAP亚组患儿年龄、性别、C反应蛋白水平、儿童危重病例评分，以及呼吸衰竭和循环衰竭比例比较差异均有统计学意义（均P<0.05），见表2。

表2　CAP 患儿的一般临床特征

项目	轻度 CAP 亚组 (n=142)	重度 CAP 亚组 (n=233)	t/χ^2 值	P 值
性别 (男/女, 例)	76/66	152/81	5.081	0.024
年龄 ($\bar{x} \pm s$, 月)	24 ± 21	42 ± 39	5.182	<0.001
PCIS (极危重/危重/非危重, 例)	0/0/142	29/28/176	40.965	<0.001
呼吸衰竭 (有/无, 例)	0/142	32/201	19.598	<0.001
循环衰竭 (有/无, 例)	0/142	17/216	9.232	0.002
C 反应蛋白 ($\bar{x} \pm s$, mg/dL)	8 ± 11	25 ± 38	5.134	<0.001
白细胞计数 ($\bar{x} \pm s$, $\times 10^9$/L)	11 ± 5	12 ± 7	1.231	0.219

注：［PCIS］儿童危重病例评分；［CAP］社区获得性肺炎。

2.2　各 TagSNPs 与儿童 CAP 的易感性及严重程度的关联分析

各位点等位基因在 CAP 组与对照组、重度 CAP 亚组与轻度 CAP 亚组之间的分布差异均无统计学意义（均 P>0.05）。*TICAM1* 基因 rs11466711T/C 位点 CC 基因型在共显性和隐性遗传模型下，在 CAP 组的分布频率均低于对照组（均 P<0.05，OR=0.577、0.568），与 CAP 易感性显著关联，考虑 CC 基因型可能为儿童 CAP 发生的保护基因型；其余各位点基因型分布在 CAP 组与对照组间差异均无统计学意义（均 P>0.05）。各位点基因型分布在重度 CAP 亚组与轻度 CAP 亚组间比较差异均无统计学意义（均 P>0.05）。见表3。

表3　各 TagSNPs 多态性与儿童 CAP 易感性及严重程度的相关性分析

基因/位点	遗传模型	基因型	易感性				严重程度			
			CAP 组 (例)	对照组 (例)	OR^*(95%CI)	P 值*	重度 CAP 亚组 (例)	轻度 CAP 亚组 (例)	OR^*(95%CI)	P 值*
TICAM1										
rs2292151G/A	共显性模型	G/G	105	89	–	–	67	38	–	–
		G/A	204	146	1.234(0.851~1.790)	0.267	124	80	0.905(0.547~1.495)	0.696
		A/A	66	71	0.783(0.495~1.241)	0.298	42	24	0.913(0.473~1.762)	0.786
	显性模型	G/G	105	89	–	–	67	38	–	–
		G/A-A/A	270	217	1.084(0.763~1.539)	0.653	166	104	0.907(0.561~1.467)	0.689
	隐性模型	G/G-G/A	309	235	–	–	191	118	–	–
		A/A	66	71	0.685(0.462~1.017)	0.061	42	24	0.975(0.552~1.721)	0.929
	加性模型	–	–	–	0.911(0.725~1.146)	0.428	–	–	0.949(0.687~1.310)	0.749
	等位基因	G	414	324	–	–	258	156	–	–
		A	336	288	0.913(0.737~1.131)	0.405	208	128	0.983(0.7301~.322)	0.907
rs7255265C/T	共显性模型	C/C	134	124	–	–	82	52	–	–
		C/T	187	140	1.297(0.919~1.832)	0.139	117	70	1.198(0.745~1.926)	0.457
		T/T	54	42	1.239(0.754~2.035)	0.397	34	20	1.149(0.585~2.258)	0.687
	显性模型	C/C	134	124	–	–	82	52	–	–
		C/T-T/T	241	182	1.284(0.926~1.780)	0.135	151	90	1.186(0.755~1.864)	0.458
	隐性模型	C/C-C/T	321	264	–	–	199	122	–	–
		T/T	54	42	1.072(0.678~1.695)	0.765	34	20	1.035(0.558~1.916)	0.914
	加性模型	–	–	–	1.157(0.915~1.462)	0.223	–	–	1.100(0.797~1.519)	0.561
	等位基因	C	455	388	–	–	281	174	–	–
		T	295	224	1.123(0.901~1.400)	0.302	185	110	1.041(0.769~1.409)	0.793
rs61231668T/C	共显性模型	T/T	91	88	–	–	57	34	–	–
		T/C	202	148	1.265(0.866~1.850)	0.225	123	79	1.075(0.633~1.825)	0.789
		C/C	82	70	1.138(0.722~1.795)	0.577	53	29	1.182(0.625~2.237)	0.607
	显性模型	T/T	91	88	–	–	57	34	–	–

表3（续）

基因/位点	遗传模型	基因型	易感性				严重程度			
			CAP组(例)	对照组(例)	*OR**(95%*CI*)	*P*值*	重度CAP亚组(例)	轻度CAP亚组(例)	*OR**(95%*CI*)	*P*值*
		T/C-C/C	284	218	1.225(0.856~1.755)	0.268	176	108	1.105(0.667~1.831)	0.697
	隐性模型	T/T-T/C	293	236	–	–	180	113	–	–
		C/C	82	70	0.975(0.666~1.428)	0.897	53	29	1.125(0.666~1.900)	0.659
	加性模型	–	–	–	1.075(0.856~1.351)	0.533	–	–	1.087(0.791~1.494)	0.607
	等位基因	T	384	324	–	–	237	147	–	–
		C	366	288	1.072(0.866~1.328)	0.522	229	137	1.037(0.772~1.393)	0.811
rs35299700C/T	共显性模型	C/C	288	244	–	–	183	105	–	–
		C/T	81	58	1.200(0.806~1.785)	0.369	46	35	0.777(0.463~1.302)	0.337
		T/T	6	4	1.584(0.420~5.980)	0.497	4	2	0.920(0.161~5.253)	0.925
	显性模型	C/C	288	244	–	–	183	105	–	–
		C/T-T/T	87	62	1.223(0.831~1.800)	0.308	50	37	0.785(0.475~1.299)	0.347
	隐性模型	C/C-C/T	369	302	–	–	229	140	–	–
		T/T	6	4	1.526(0.405~5.746)	0.532	4	2	0.974(0.171~5.538)	0.976
	加性模型	–	–	–	1.214(0.856~1.722)	0.277	–	–	0.819(0.520~1.290)	0.390
	等位基因	C	657	546	–	–	412	245	–	–
		T	93	66	1.171(0.838~1.637)	0.356	54	39	0.823(0.529~1.280)	0.387
rs6510826G/A	共显性模型	G/G	97	97	–	–	55	42	–	–
		G/A	190	145	1.337(0.921~1.940)	0.127	121	69	1.315(0.787~2.198)	0.296
		A/A	87	64	1.360(0.869~2.129)	0.179	56	31	1.426(0.773~2.629)	0.256
	显性模型	G/G	97	97	–	–	55	42	–	–
		G/A-A/A	277	209	1.344(0.947~1.906)	0.098	177	100	1.349(0.832~2.189)	0.225
	隐性模型	G/G-G/A	287	242	–	–	176	111	–	–
		A/A	87	64	1.135(0.773~1.665)	0.518	56	31	1.194(0.714~1.997)	0.500
	加性模型	–	–	–	1.178(0.941~1.473)	0.152	–	–	1.200(0.883~1.630)	0.245
	等位基因	G	384	339	–	–	231	153	–	–
		A	364	273	1.177(0.950~1.459)	0.136	233	131	1.178(0.876~1.584)	0.278
rs11466711T/C	共显性模型	T/T	153	119	–	–	95	58	–	–
		T/C	181	136	1.030(0.730 ~1.453)	0.868	110	71	0.898(0.569~1.416)	0.642
		C/C	41	51	0.577(0.351~0.949)	0.030	28	13	1.247(0.588~2.643)	0.565
	显性模型	T/T	153	119	–	–	95	58	–	–
		T/C-C/C	222	187	0.901(0.652~1.247)	0.531	138	84	0.952(0.615~1.474)	0.826
	隐性模型	T/T-T/C	334	255	–	–	205	129	–	–
		C/C	41	51	0.568(0.358~0.902)	0.016	28	13	1.322(0.651~2.686)	0.440
	加性模型	–	–	–	0.821(0.650~1.036)	0.097	–	–	1.035(0.746~1.436)	0.838
	等位基因	T	487	374	–	–	300	187	–	–
		C	263	238	0.849(0.680~1.059)	0.146	166	97	1.067(0.782~1.454)	0.683
rs10422141A/T	共显性模型	A/A	207	172	–	–	136	71	–	–
		A/T	147	115	1.115(0.797~1.559)	0.525	85	62	0.730(0.465~1.146)	0.171
		T/T	21	19	0.843(0.428~1.661)	0.622	12	9	0.666(0.259~1.708)	0.398
	显性模型	A/A	207	172	–	–	136	71	–	–
		A/T-T/T	168	134	1.073(0.778~1.480)	0.666	97	71	0.721(0.467~1.115)	0.141
	隐性模型	A/A-A/T	354	287	–	–	221	133	–	–
		T/T	21	19	0.806(0.415~1.565)	0.524	12	9	0.763(0.304~1.916)	0.565
	加性模型	–	–	–	1.014(0.779~1.318)	0.919	–	–	0.770(0.538~1.100)	0.151

表3（续）

基因/位点	遗传模型	基因型	易感性				严重程度			
			CAP组(例)	对照组(例)	*OR**(95%*CI*)	*P*值*	重度CAP亚组(例)	轻度CAP亚组(例)	*OR**(95%*CI*)	*P*值*
	等位基因	A	561	459	–	–	357	204	–	–
		T	189	153	1.011(0.790~1.293)	0.933	109	80	0.779(0.556~1.089)	0.144
rs35747610G/A	共显性模型	G/G	243	209	–	–	153	90	–	–
		G/A	119	90	1.138(0.803~1.611)	0.466	73	46	0.938(0.588~1.495)	0.787
		A/A	13	7	1.808(0.678~4.820)	0.236	7	6	0.632(0.199~1.997)	0.434
	显性模型	G/G	243	209	–	–	153	90	–	–
		G/A-A/A	132	97	1.184(0.845~1.659)	0.327	80	52	0.901(0.575~1.412)	0.648
	隐性模型	G/G-G/A	362	299	–	–	226	136	–	–
		A/A	13	7	1.734(0.655~4.594)	0.268	7	6	0.645(0.206~2.019)	0.452
	加性模型	–	–	–	1.201(0.893~1.615)	0.226	–	–	0.881(0.599~1.296)	0.520
	等位基因	G	605	508	–	–	379	226	–	–
		A	145	104	1.171(0.887~1.546)	0.266	87	58	0.895(0.617~1.296)	0.555
MyD88										
rs7744A/G	共显性模型	A/A	174	120	–	–	106	68	–	–
		A/G	164	149	0.764(0.545~1.071)	0.118	104	60	1.020(0.646~1.610)	0.933
		G/G	37	37	0.767(0.449~1.321)	0.343	23	14	1.001(0.472~2.124)	0.997
	显性模型	A/A	174	120	–	–	106	68	–	–
		A/G-G/G	201	186	0.765(0.555~1.056)	0.103	127	74	1.016(0.659~1.568)	0.942
	隐性模型	A/A-A/G	338	269	–	–	210	128	–	–
		G/G	37	37	0.886(0.532~1.475)	0.641	23	14	0.992(0.483~2.038)	0.983
	加性模型	–	–	–	0.837(0.658~1.066)	0.149	–	–	1.008(0.725~1.400)	0.964
	等位基因	A	512	389	–	–	316	196	–	–
		G	238	223	0.811(0.647~1.016)	0.068	150	88	1.057(0.769~1.453)	0.731

注：*组间等位基因进行χ^2检验分析，组间基因型进行logistic回归分析，并使用性别、年龄等显著环境因子校正后的逻辑回归分析值。［CAP］社区获得性肺炎。

2.3 各 TagSNPs 与儿童 CAP 临床特征的关联分析

*MyD88*基因rs7744A/G位点GG基因型在隐性和共显性纯合遗传模型下与CAP患儿呼吸衰竭、循环衰竭关联，能显著增加患儿发生呼吸衰竭及循环衰竭的风险（均*P*<0.05）；*TICAM1*基因rs6510826G/A位点AA基因型在隐性、加性和共显性/纯合遗传模型下与患儿C反应蛋白水平密切关联（均*P*<0.05），携带该基因型的患儿C反应蛋白水平增高更为显著；*TICAM1*基因rs35747610G/A位点AA基因型在隐性和共显性纯合遗传模型下均与CAP患儿并发脓毒症的风险密切关联（均*P*<0.05），携带该基因型可显著降低CAP患儿并发脓毒症的风险。见表4。

2.4 *MyD88*和*TICAM1*基因间交互作用与儿童CAP的关联分析

*MyD88*基因rs7744A/G与*TICAM1*基因rs11466711T/C位点之间存在与CAP易感性显著关联的正相乘模型交互作用（*P*<0.05，*OR*=1.420），即当上述两交互位点（rs7744-rs11466711）的基因型均为纯合风险基因型（GG-CC）时，CAP的发生风险比基因型均为纯合野生基因型（AA-TT）显著增加；*MyD88*基因rs7744A/G分别与*TICAM1*基因rs35299700C/T及rs35747610G/A位点之间存在与CAP严重程度相关联的正相乘模型交互作用（均*P*<0.05，*OR*=2.508、2.551），即当上述交互的两位点均变异为纯合风险基因型（GG-TT、GG-AA）时，CAP患儿发生重症肺炎的风险比两位点同时

携带纯合野生基因型（AA-CC、AA-GG）显著增加；同样，*MyD88*基因rs7744A/G分别与*TICAM1*基因rs2292151G/A及rs35747610G/A位点之间存在与CAP并发脓毒症风险相关联的正相乘模型交互作用（均*P*<0.05，*OR*=1.451、2.318），即当rs7744与rs2292151和rs35747610的交互基因型均为纯合风险基因型组合（GG-AA）时，患儿并发脓毒症的风险较均为纯合野生基因型组合（AA-GG）显著增加。见表5。

表4　临床特征关联分析中具有显著统计学意义的关联位点

临床特征	位点	遗传模型	基因型	*Beta**	*SE*	95%*CI*下限	95%*CI*上限	*STAT*值	*P*值*
TICAM1									
C反应蛋白	rs6510826G/A	隐性模型	GG-GA/AA	8.060	3.900	0.417	15.700	2.067	0.039
		共显性纯合模型	GG/AA	10.620	4.688	1.431	19.810	2.265	0.024
		加性模型	–	5.266	2.339	0.682	9.850	2.251	0.025
脓毒症	rs35747610G/A	隐性模型	GG-GA/AA	–0.429	0.183	–0.787	–0.071	–2.350	0.019
		共显性纯合模型	GG/AA	–0.427	0.184	–0.788	–0.066	–2.318	0.021
MyD88									
呼吸衰竭	rs7744A/G	隐性模型	AA-AG/GG	0.322	0.474	0.127	0.816	–2.389	0.017
		共显性纯合模型	AA/GG	0.305	0.520	0.110	0.844	–2.286	0.022
循环衰竭	rs7744A/G	隐性模型	AA-AG/GG	0.238	0.568	0.078	0.724	–2.529	0.011
		共显性纯合模型	AA/GG	0.233	0.640	0.066	0.816	–2.277	0.023

注：*连续型变量使用线性回归进行分析，分类变量使用逻辑回归进行分析，并使用性别、年龄等显著环境因子校正。

表5　*MyD88*与*TICAM1*基因交互作用与CAP的关联分析

交互作用变量	基因型	CAP		重度CAP		脓毒症	
MyD88×*TICAM1*		*OR*(95%*CI*)	*P*	*OR*(95%*CI*)	*P*	*OR*(95%*CI*)	*P*
rs7744×rs10422141	AA-AA/GG-TT	1.279(0.878~1.864)	0.199	0.991(0.582~1.688)	0.974	1.294(0.762~2.198)	0.340
rs7744×rs11466711	AA-TT/GG-CC	1.420(1.017~1.983)	0.039	1.430(0.865~2.364)	0.163	1.035(0.641~1.672)	0.888
rs7744×rs2292151	AA-GG/GG-AA	0.937(0.669~1.312)	0.705	1.142(0.694~1.878)	0.600	1.451(1.008~2.089)	0.045
rs7744×rs35299700	AA-CC/GG-TT	1.270(0.773~2.085)	0.345	2.508(1.273~4.941)	0.008	1.338(0.662~2.705)	0.417
rs7744×rs35747610	AA-GG/GG-AA	1.393(0.911~2.129)	0.126	2.551(1.184~5.496)	0.017	2.318(1.205~4.460)	0.012
rs7744×rs61231668	AA-TT/GG-CC	0.950(0.682~1.324)	0.763	0.941(0.577~1.535)	0.808	0.997(0.617~1.609)	0.990
rs7744×rs6510826	AA-GG/GG-AA	1.029(0.744~1.422)	0.863	0.678(0.465~1.081)	0.102	0.797(0.506~1.253)	0.325
rs7744×rs7255265	AA-CC/GG-TT	0.863(0.609~1.220)	0.403	0.885(0.536~1.461)	0.633	1.035(0.641~1.672)	0.888

注：［CAP］社区获得性肺炎。

3　讨论

MyD88是TLRs信号通路中MyD88依赖途径的关键衔接蛋白，阻断MyD88表达可以减轻脓毒症的炎性因子分泌，对脓毒症诱导的心肌损伤具有强大的保护作用[10]。Kohl等[11]发现，*Myd88*$^{-/-}$小鼠可以显著降低病原诱导的炎性细胞因子和干扰素-γ诱导基因的表达水平。*MyD88*基因rs7744位点位于3'UTR非编码区，该区域与mRNA的稳定性和蛋白表达相关。孙丹丹等[12]发现，rs7744多态性与冠心病的发病风险及严重程度密切关联。Jiménez-Sousa等[13]发现，rs7744位点GG基因型与败血症休克患者的死亡风险相关。本研究发现，*MyD88*基因rs7744位点GG基因型与CAP患儿并发呼吸衰竭和循环衰竭的风险显著关联，推测该关联可能与*MyD88*基因rs7744多态性影响*MyD88*-NF-κB通路活性及下游的炎性基因表达有关，至于该位点的多态性与CAP重症指标呼吸衰竭和循环衰竭相关，却与儿童CAP严重程度无关的原因，还需要进一步探讨。

TICAM1是TLRs信号通路中TICAM1依赖途径的关键衔接蛋白，其基因多态性与多种疾病密切

相关[14-15]。本研究发现，*TICAM1*基因rs11466711位点多态性与儿童CAP易感性有关，该位点CC基因型可能为儿童CAP发生的保护基因型，并且该基因rs6510826位点多态性与患儿C反应蛋白水平相关联，该位点携带AA基因型的患儿肺部炎症反应更为强烈。*TICAM1*基因rs11466711和rs6510826是位于第1内含子区域上的2个非编码SNPs，并不会直接参与蛋白的合成，但是内含子的变异可产生剪切变异并影响基因的转录，上述两位点多态性对CAP患儿病情发展产生的影响并不相同，考虑与转录过程中可能存在的选择性剪切有关。研究表明，TLRs通路相关基因多态性与脓毒症的发生、发展及预后密切相关[16]。本研究发现，*TICAM1*基因rs35747610位点AA基因型可显著降低CAP患儿并发脓毒症的风险。该位点是处于基因间区域5'UTR上游20 kb范围内的1个位点，有研究[17]认为，该区域范围内的SNPs可能会影响靶基因miRNA的表达水平，并参与了脓毒症的病理生理机制，与脓毒症患者的临床表现和炎性反应密切相关，故推测，该关联可能与*TICAM1*基因rs35747610位点多态性影响*TICAM1*基因miRNA的表达及相关免疫通路的活性有关。

*TICAM1*基因rs2292151和rs7255265位点是2个位于第2外显子上的功能位点，均为同义SNPs，虽然同义SNP不改变编码的氨基酸，但却会改变剪切体的亲和性从而影响剪切。Wang等[18]发现，*TICAM1*基因rs2292151多态性改变了TICAM1的作用，并参与了肿瘤坏死因子和Ⅰ型干扰素合成的调控及先天免疫信号的转导和激活过程。Cheng等[14]分析显示，与*TICAM1*基因rs7255265位点高度连锁（均r^2=0.919）的rs4807000和rs6510827位点参与*TICAM1*基因的表达调控，并与中国汉族人群白癜风风险显著相关。Sahiner等[19]研究显示，rs4807000位点多态性影响内毒素对体外IgE合成的调控，与哮喘患儿气道高反应性及喘息密切相关。但本研究分析结果却并未发现这2个外显子位点与CAP病情发展相关联。对于这种分析结果，多认为可能与所研究人群的地域、种族及疾病种类不同有关，但是这种解释似乎并不完全。

目前已经发现的遗传因素尚不足以完全解释疾病的遗传度，研究认为，基因交互作用和微效基因与这些“丢失的遗传度”有关[20]。众多研究也证实，基因交互作用与疾病风险显著相关[21-22]。本研究发现，*MyD88*和*TICAM1*基因之间存在着多个与CAP易感性、严重程度及并发脓毒症显著关联的相乘交互作用模式。那些在SNPs关联分析中未被发现但实际存在的微效基因位点，由于其单个位点变异不足以影响机体整个炎症反应强度，故而无法在SNPs关联分析中发现，但这些微效位点却在交互作用中表现出强大的协同作用。上述结果同时表明，*MyD88*和*TICAM1*基因均参与了儿童CAP的免疫炎症调控，并协同影响CAP的病情进展。

综上所述，*MyD88*和*TICAM1*基因存在多个与儿童CAP相关的功能性变异，并且这些变异对儿童CAP的病情发展具有协同作用。这些研究为今后开展CAP患儿个体化治疗和重症CAP的预防提供了潜在的靶点，在儿童CAP的临床诊疗实践中，如何通过沉默或拮抗来调控*MyD88*和*TICAM1*基因的表达，协调TLRs信号通路内不同信号途径的平衡，均是今后研究有待解决的问题和面临的挑战。

利益冲突声明：所有作者均声明不存在任何利益冲突。

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Chinese version

Association of gene polymorphisms of *MyD88* and *TICAM1* and their interactions with community-acquired pneumonia in children*

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Abstract: Objective To investigate the association of single nucleotide polymorphisms (SNPs) of myeloid differentiation factor 88 (*MyD88*) and Toll-like receptor adaptor molecule 1 (*TICAM1*) and their interactions with community-acquired pneumonia (CAP) in children. **Methods** Improved multiple ligase detection reaction assay was used for detecting the polymorphisms of nine tagging SNPs of the *MyD88* and *TICAM1* genes in 375 children with CAP who attended the Department of Pediatrics of the Second Affiliated Hospital of Yan'an University Medical School from August 2015 to September 2017 and 306 healthy children who underwent physical examination. A logistic regression analysis was used to evaluate the association between the distribution of genotypes and their interactions with CAP in children. **Results** The polymorphism of the *TICAM1* gene at rs11466711T/C locus was closely associated with the susceptibility to CAP in children (P<0.05). The AA genotype of rs35747610G/A locus significantly reduced risk of sepsis in children with CAP (P<0.05). The AA genotype of rs6510826G/A locus was significantly associated with the increase in C-reactive protein level in children with CAP (P<0.05). The GG genotype of the *MyD88* gene at rs7744A/G locus significantly increased the risk of respiratory failure and circulatory failure (P<0.05). The multiplicative interactions between *MyD88* gene rs7744A/G and *TICAM1* gene rs11466711T/C, rs2292151G/A, rs35299700C/T, and rs35747610G/A loci were significantly associated with the susceptibility to CAP, the severity of CAP, and the risk of sepsis in children (P<0.05). **Conclusions** The gene polymorphisms of *MyD88* and *TICAM1* and their interactions are closely associated with CAP in children, with a synergistic effect on the development and progression of CAP in children.

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Key words: Community-acquired pneumonia; *MyD88*; *TICAM1*; Gene polymorphism; Child

Community-acquired pneumonia (CAP) is one of the most common serious infections in children. Molecular epidemiological studies have demonstrated that CAP is resulted by combinations between host genetic and environmental factors[1]. The exact mechanism oflung injury is complex, and involves a large number of cellular and molecular immune processes[2-3]. Toll-like receptors (TLRs) signaling pathway plays an important role in the process. TLRs signaling pathway is divided into two pathways: one is myeloid differentiation factor 88 (MyD88) pathways, another is Toll-like receptor adaptor molecule 1 (TICAM1) pathway, which plays an important role in the either early or late stages of bacterial lipopolysaccharide-mediated nuclear factor-κB activation, respectively. Thus, the TLRs signaling pathway is an important pathway for the activation and amplification of inflammatory reactions, and plays a key role in the outcome of infection[4]. The single nucleotide polymorphisms (SNPs) of *TLRs*

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gene has been shown to affect the susceptibility of CAP[5-6]. However, the association of gene polymorphisms of *MyD88* and *TICAM1* with CAP in children, as well as the role of their mediated related immune pathways in CAP are not clear. Therefore, in this study, nine tagging SNPs (TagSNPs) of *MyD88* and *TICAM1* were selected to explore association between the SNPs and CAP in children. The results of our research will be used in strategies development and potential targets for CAP treatment in children.

1 Materials and methods

1.1 Study subjects

A total of 375 hospitalized children, 228 males and 147 females, aged >28 d and ≤15 years with an average age of (38±22) months, at the Department of Pediatrics, the Second Affiliated Hospital of Yan'an University Medical School from August 2015 to September 2017 were prospectively selected. All children met the diagnostic criteria of the Guidelines for the Management of Community-acquired Pneumonia in Children revised by the Respiratory Group of Chinese Pediatric Society of Chinese Medical Association[7]. The 375 patients were further divided into severe CAP subgroup (233 cases) and mild CAP subgroup (142 cases). According to the diagnosis and grading criteria of Extracorporeal Life Support Organization (ELSO) Guidelines for Pediatric Respiratory Failure[8], the 375 patients were classified into non-sepsis subgroup (175 cases), mild sepsis subgroup (163 cases) and severe sepsis subgroup (37 cases, including children with septic shock). Among the 375 patients, 32 cases were complicated with respiratory failure and 17 cases with circulatory failure. The diagnostic criteria for respiratory failure were based on the relevant criteria for pediatric respiratory failure issued by Extracorporeal Life Support Organization[8]. The diagnosis of circulatory failure was based on the international guidelines for management of severe sepsis and septic shock[9]. Exclusion criteria of CAP group: (1) autoimmune diseases or acquired immunodeficiency disease; (2) tumor; (3) asthma; (4) congenital heart and lung disease; (5) hereditary, metabolic diseases; (6) combined with cerebral palsy, central coordination disorder; (7) hospital-acquired severe pneumonia. The control group consisted of 306 children who underwent health examination in the child health clinic during the same period, including 183 males and 123 females, with an average age of (38±24) months. None of the children in the control group had a history of pneumonia or severe infectious diseases before blood samples were submitted for examination, and the exclusion criteria were referred to the CAP group.

Both the two groups were unrelated Han Chinese populations in northern Shaanxi, and no significant difference in terms of age (χ^2=0.069, P=0.688) and sex (t=0.402, P=0.792). All children were enrolled with parental consent and signed an informed consent form. This research project was approved by the Ethics Committee of the Second Affiliated Hospital of Yan'an University Medical School (YLYY201505081).

1.2 Selection of TagSNPs in candidate genes

Using NCBI-SNP (http://www.ncbi.nlm.nih.gov/gen) and 1000 Genomes (http://browser.1000genomes.org/Homo_sapiens/UserData/Haploview) database downloads the location information of candidate genes in the Han population of southern China and the Han population of Beijing, China (version GRCh37), and then selects TagSNPs loci using Haploview 4.2 software. The inclusion criteria were that the position information was enlarged by 2 kb from upstream of the promoter to before and after the end of the gene, the linkage disequilibrium parameter D' =1, the linkage disequilibrium coefficient r^2 ≥0.8 and the minimum allele frequency ≥0.1, and a total of 9 TagSNPs were selected for the study in the *MyD88* and *TICAM1* genes, loci information is shown in Table 1. In this study, the success rate of SNPs typing was 99.9%-100%. The genotype distribution frequency of 9 TagSNPs in the control group was consistent with the Hardy-Weinberg equilibrium test (all P>0.05). See Table 1.

Table 1 Information, functional prediction of the TagSNPs and Hardy-Weinberg equilibrium analysis in the control group

Gene/locus	Location area	Predictive feature	MAF in the control group	Genotype frequency (11/01/00)	HWE in the control group (*P* value)
TICAM1					
rs2292151G/A	Exon 2	p.=(Asp557Asp)	0.471	71/146/89	0.491
rs7255265C/T	Exon 2	p.=(Thr4Thr)	0.366	42/140/124	0.806
rs61231668T/C	Intron 1	–	0.471	70/148/88	0.646
rs35299700C/T	Intron 1	–	0.108	4/58/244	0.765
rs6510826G/A	Intron 1	–	0.446	64/145/97	0.488
rs11466711T/C	Intron 1	–	0.389	51/136/119	0.279
rs10422141A/T	5' upstream intergenic region	dist=1 656 bp	0.250	19/115/172	1.000
rs35747610G/A	5' upstream intergenic region	dist=1 867 bp	0.170	7/90/209	0.547
MyD88					
rs7744A/G	3' non-coding region	–	0.364	37/149/120	0.459

Note: [MAF] minor allele frequency; [HWE] Hardy-Weinberg equilibrium; [11/01/00] homozygous low-frequency allele/heterozygous/homozygous high-frequency allele; [dist] distance from 5' UTR of *TICAM*1 gene.

1.3 Blood sample collection and DNA extraction

Two milliliters of venous blood were collected in tubes containing anticoagulant (ethylenediaminetetraacetic acid) from children on the day of admission, and temporarily stored at −20℃ for future use. Anticoagulated whole blood genomic DNA was extracted using the DNA extraction kit from Omega, USA. The obtained DNA samples were subjected to quality inspection and concentration detection using 1% agarose electrophoresis, and then diluted to a working concentration of 10 ng/μL, and placed in a −20℃ freezer for cryopreservation.

1.4 Gene polymorphism detection

A total of nine TagSNPs loci in 681 samples were genotyped using a modified multiplex high-temperature ligase detection reaction technique developed by Shanghai Tianhao Biotechnology Co., Ltd. According to the gene sequence on GenBank, the primers were designed using Primer 5.0 software. And the primers were designed and synthesized by Shanghai Sangon Biological Co., Ltd. In this study, DNA extraction, polymerase chain reaction amplification of DNA, ligation reaction detection and original data analysis were completed by Shanghai Tianhao Biotechnology Co., Ltd., sequenced using ABI 3730XL sequencer (ABI, USA), and genotypes were interpreted using GeneMapper 4.1 software (ABI, USA).

1.5 Statistical analysis

Hardy-Weinberg equilibrium analysis of the control group was estimated with the PLINK software. Statistical analysis was performed using SPSS 20.0 software, and $P<0.05$ indicated a statistically significant difference. Normally distributed measurement data were presented as mean ± standard deviation ($\bar{x} \pm s$), and two-sample t tests were used to compare groups; enumeration data are expressed as number of cases, and chi-square test was used for comparison between groups. The association between genotype and CAP was analyzed by logistic regression, and the effects of significant confounders such as gender, age and living environment were adjusted, and the analyzed genetic models were dominant model, recessive model, additive model and codominant homozygous/heterozygous model. Intergenic interactions were analyzed using logistic regression models, statistical models for interactions using multiplicative models, and models for alleles as additive models. Genotypes were used as variables, and the values were assigned according to the number of risk alleles. The interaction terms of SNPs in the two genes were included in the logistic regression model and the regression coefficients were hypothesis tested. The interaction results were determined using the Rothman multiplicative interaction evaluation formula, that is, the 95%*CI* of the product term of the

logistic regression did not contain 1, indicating that there was a multiplicative interaction, $OR_{A\times B}$=1 was no interaction; $OR_{A\times B}$>1 was a positive interaction; $OR_{A\times B}$<1 was a negative interaction.

2 Results

2.1 Comparison of general information of children with CAP

There were significant differences in age, gender, C-reactive protein level, critical illness score, and proportion of respiratory failure and circulatory failure between children with severe CAP and those with mild CAP (all P<0.05) (Table 2).

2.2 Association analysis of the TagSNPs with susceptibility and severity of CAP in children

There were no significant differences in the distribution of alleles between the CAP group and the control group, the severe CAP subgroup and the mild CAP subgroup (all P>0.05). However, the CC genotype distribution for rs11466711T/C in the *TICAM1* gene was less frequent in the CAP group than in the control group under both codominant and recessive genetic models (all P<0.05, OR=0.577, 0.568). The result showed a significantly association with CAP susceptibility, and indicated that the CC genotype may be a protective genotype for the development of CAP in children. There were no significant differences in the genotype distribution at other loci between the CAP group and the control group (all P>0.05). Moreover, there were no significant differences in genotype distribution between the severe CAP subgroup and the mild CAP subgroup (all P>0.05). See Table 3.

Table 2 General clinical characteristics of children with CAP

Item	Mild CAP subgroup (n=142)	Severe CAP subgroup (n=233)	t/χ^2 value	P value
Gender (male/female, case)	76/66	152/81	5.081	0.024
Age ($\bar{x}\pm s$, month)	24±21	42±39	5.182	<0.001
PCIS (very critical/critical/non-critical, case)	0/0/142	29/28/176	40.965	<0.001
Respiratory failure (yes/no, case)	0/142	32/201	19.598	<0.001
Circulatory failure (yes/no, case)	0/142	17/216	9.232	0.002
C-reactive protein ($\bar{x}\pm s$, mg/dL)	8±11	25±38	5.134	<0.001
White blood cell count ($\bar{x}\pm s$, $\times10^9$/L)	11±5	12±7	1.231	0.219

Note: [PCIS] pediatric critical illness score; [CAP] community-acquired pneumonia.

Table 3 Association analysis of the TagSNPs polymorphism with susceptibility and severity of CAP in children

Gene/locus	Heredity model	Genotype	Susceptibility				Severity			
			CAP group (n)	Control group (n)	OR^*(95%CI)	P value*	Severe CAP subgroup (n)	Mild CAP subgroup (n)	OR^*(95%CI)	P value*
TICAM1										
rs2292151G/A	Codominant model	G/G	105	89	–	–	67	38	–	–
		G/A	204	146	1.234(0.851-1.790)	0.267	124	80	0.905(0.547-1.495)	0.696
		A/A	66	71	0.783(0.495-1.241)	0.298	42	24	0.913(0.473-1.762)	0.786
	Dominant model	G/G	105	89	–	–	67	38	—	–
		G/A-A/A	270	217	1.084(0.763-1.539)	0.653	166	104	0.907(0.561-1.467)	0.689
	Recessive model	G/G-G/A	309	235	–	–	191	118	–	–

Table 3 (continued)

Gene/locus	Heredity model	Genotype	Susceptibility				Severity			
			CAP group (*n*)	Control group (*n*)	*OR**(95%*CI*)	*P* value*	Severe CAP subgroup (*n*)	Mild CAP subgroup (*n*)	*OR**(95%*CI*)	*P* value*
		A/A	66	71	0.685(0.462-1.017)	0.061	42	24	0.975(0.552-1.721)	0.929
	Additive model	–	–	–	0.911(0.725-1.146)	0.428	–	–	0.949(0.687-1.310)	0.749
	Allelic model	G	414	324	–	–	258	156	–	–
		A	336	288	0.913(0.737-1.131)	0.405	208	128	0.983(0.7301-322)	0.907
rs7255265C/T	Codominant model	C/C	134	124	–	–	82	52	–	–
		C/T	187	140	1.297(0.919-1.832)	0.139	117	70	1.198(0.745-1.926)	0.457
		T/T	54	42	1.239(0.754-2.035)	0.397	34	20	1.149(0.585-2.258)	0.687
	Dominant model	C/C	134	124	–	–	82	52	–	–
		C/T-T/T	241	182	1.284(0.926-1.780)	0.135	151	90	1.186(0.755-1.864)	0.458
	Recessive model	C/C-C/T	321	264	–	–	199	122	–	–
		T/T	54	42	1.072(0.678~1.695)	0.765	34	20	1.035(0.558-1.916)	0.914
	Additive model	–	–	–	1.157(0.915-1.462)	0.223	-	-	1.100(0.797-1.519)	0.561
	Allelic model	C	455	388	–	–	281	174	–	–
		T	295	224	1.123(0.901~1.400)	0.302	185	110	1.041(0.769-1.409)	0.793
rs61231668T/C	Codominant model	T/T	91	88	–	–	57	34	–	–
		T/C	202	148	1.265(0.866-1.850)	0.225	123	79	1.075(0.633-1.825)	0.789
		C/C	82	70	1.138(0.722-1.795)	0.577	53	29	1.182(0.625-2.237)	0.607
	Dominant model	T/T	91	88	–	–	57	34	–	–
		T/C-C/C	284	218	1.225(0.856-1.755)	0.268	176	108	1.105(0.667-1.831)	0.697
	Recessive model	T/T-T/C	293	236	–	–	180	113	–	–
		C/C	82	70	0.975(0.666-1.428)	0.897	53	29	1.125(0.666-1.900)	0.659
	Additive model	–	–	–	1.075(0.856-1.351)	0.533	–	–	1.087(0.791-1.494)	0.607
	Allelic model	T	384	324	–	–	237	147	–	–
		C	366	288	1.072(0.866-1.328)	0.522	229	137	1.037(0.772-1.393)	0.811
rs35299700C/T	Codominant model	C/C	288	244	–	–	183	105	–	–
		C/T	81	58	1.200(0.806-1.785)	0.369	46	35	0.777(0.463-1.302)	0.337
		T/T	6	4	1.584(0.420-5.980)	0.497	4	2	0.920(0.161-5.253)	0.925
	Dominant model	C/C	288	244	–	–	183	105	–	–
		C/T-T/T	87	62	1.223(0.831-1.800)	0.308	50	37	0.785(0.475-1.299)	0.347
	Recessive model	C/C-C/T	369	302	–	-	229	140	–	–
		T/T	6	4	1.526(0.405-5.746)	0.532	4	2	0.974(0.171-5.538)	0.976
	Additive model	–	–	–	1.214(0.856-1.722)	0.277	–	–	0.819(0.520-1.290)	0.390
	Allelic model	C	657	546	–	–	412	245	–	–

Table 3 (continued)

Gene/locus	Heredity model	Genotype	Susceptibility				Severity			
			CAP group (*n*)	Control group (*n*)	*OR**(95%*CI*)	*P* value*	Severe CAP subgroup (*n*)	Mild CAP subgroup (*n*)	*OR**(95%*CI*)	*P* value*
		T	93	66	1.171(0.838-1.637)	0.356	54	39	0.823(0.529-1.280)	0.387
rs6510826G/A	Codominant model	G/G	97	97	–	–	55	42	–	–
		G/A	190	145	1.337(0.921-1.940)	0.127	121	69	1.315(0.787-2.198)	0.296
		A/A	87	64	1.360(0.869-2.129)	0.179	56	31	1.426(0.773-2.629)	0.256
	Dominant model	G/G	97	97	–	–	55	42	–	–
		G/A-A/A	277	209	1.344(0.947-1.906)	0.098	177	100	1.349(0.832-2.189)	0.225
	Recessive model	G/G-G/A	287	242	–	–	176	111	–	–
		A/A	87	64	1.135(0.773-1.665)	0.518	56	31	1.194(0.714-1.997)	0.500
	Additive model	–	–	–	1.178(0.941-1.473)	0.152	–	–	1.200(0.883-1.630)	0.245
	Allelic model	G	384	339	–	–	231	153	–	–
		A	364	273	1.177(0.950-1.459)	0.136	233	131	1.178(0.876-1.584)	0.278
rs11466711T/C	Codominant model	T/T	153	119	–	–	95	58	–	–
		T/C	181	136	1.030(0.730-1.453)	0.868	110	71	0.898(0.569-1.416)	0.642
		C/C	41	51	0.577(0.351-0.949)	0.030	28	13	1.247(0.588-2.643)	0.565
	Dominant model	T/T	153	119	–	–	95	58	–	–
		T/C-C/C	222	187	0.901(0.652-1.247)	0.531	138	84	0.952(0.615-1.474)	0.826
	Recessive model	T/T-T/C	334	255	–	–	205	129	–	–
		C/C	41	51	0.568(0.358-0.902)	0.016	28	13	1.322(0.651-2.686)	0.440
	Additive model	–	–	–	0.821(0.650-1.036)	0.097	–	–	1.035(0.746-1.436)	0.838
	Allelic model	T	487	374	–	–	300	187	–	–
		C	263	238	0.849(0.680-1.059)	0.146	166	97	1.067(0.782-1.454)	0.683
rs10422141A/T	Codominant model	A/A	207	172	–	–	136	71	–	–
		A/T	147	115	1.115(0.797-1.559)	0.525	85	62	0.730(0.465-1.146)	0.171
		T/T	21	19	0.843(0.428-1.661)	0.622	12	9	0.666(0.259-1.708)	0.398
	Dominant model	A/A	207	172	–	–	136	71	–	–
		A/T-T/T	168	134	1.073(0.778-1.480)	0.666	97	71	0.721(0.467-1.115)	0.141
	Recessive model	A/A-A/T	354	287	–	–	221	133	–	–
		T/T	21	19	0.806(0.415-1.565)	0.524	12	9	0.763(0.304-1.916)	0.565
	Additive model	–	–	–	1.014(0.779-1.318)	0.919	-	-	0.770(0.538-1.100)	0.151
	Allelic model	A	561	459	–	–	357	204	–	–
		T	189	153	1.011(0.790-1.293)	0.933	109	80	0.779(0.556-1.089)	0.144
rs35747610G/A	Codominant model	G/G	243	209	–	–	153	90	–	–

Table 3 (continued)

Gene/locus	Heredity model	Genotype	Susceptibility				Severity			
			CAP group (*n*)	Control group (*n*)	*OR**(95%*CI*)	*P* value*	Severe CAP subgroup (*n*)	Mild CAP subgroup (*n*)	*OR**(95%*CI*)	*P* value*
		G/A	119	90	1.138(0.803-1.611)	0.466	73	46	0.938(0.588-1.495)	0.787
		A/A	13	7	1.808(0.678-4.820)	0.236	7	6	0.632(0.199-1.997)	0.434
	Dominant model	G/G	243	209	–	–	153	90	–	–
		G/A-A/A	132	97	1.184(0.845-1.659)	0.327	80	52	0.901(0.575-1.412)	0.648
	Recessive model	G/G-G/A	362	299	–	–	226	136	–	–
		A/A	13	7	1.734(0.655-4.594)	0.268	7	6	0.645(0.206-2.019)	0.452
	Additive model	–	–	–	1.201(0.893-1.615)	0.226	–	–	0.881(0.599-1.296)	0.520
	Allele model	G	605	508	–	–	379	226	–	–
		A	145	104	1.171(0.887-1.546)	0.266	87	58	0.895(0.617-1.296)	0.555
MyD88										
rs7744A/G	Codominant model	A/A	174	120	–	–	106	68	–	–
		A/G	164	149	0.764(0.545-1.071)	0.118	104	60	1.020(0.646-1.610)	0.933
		G/G	37	37	0.767(0.449-1.321)	0.343	23	14	1.001(0.472-2.124)	0.997
	Dominant model	A/A	174	120	–	–	106	68	–	–
		A/G-G/G	201	186	0.765(0.555-1.056)	0.103	127	74	1.016(0.659-1.568)	0.942
	Recessive model	A/A-A/G	338	269	–	–	210	128	–	–
		G/G	37	37	0.886(0.532-1.475)	0.641	23	14	0.992(0.483-2.038)	0.983
	Additive model	–	–	–	0.837(0.658-1.066)	0.149	–	–	1.008(0.725-1.400)	0.964
	Allelic model	A	512	389	–	–	316	196	–	–
		G	238	223	0.811(0.647-1.016)	0.068	150	88	1.057(0.769-1.453)	0.731

Note: * χ^2 test was used for allelic model, genotyping was analyzed by logistic regressions and the values were analyzed using logistic regressions adjusted for significant environmental factors such as sex and age. [CAP] community-acquired pneumonia.

2.3 Association of the TagSNPs with clinical characteristics of CAP in children

The GG genotype of the *MyD88* gene at rs7744A/G was associated with respiratory failure and circulatory failure in children with CAP under recessive and codominant homozygous genetic models and could significantly increase the risk of the diseases process (all $P<0.05$). The AA genotype of the *TICAM1* gene at rs6510826G/A was closely associated with a higher C-reactive protein levels in children under recessive, additive, and codominant/homozygous genetic models (all $P<0.05$). The AA genotype of the *TICAM1* gene at rs35747610G/A was closely associated with a lower risk of concurrent sepsis in children with CAP under both recessive and codominant homozygous genetic models (all $P<0.05$). See Table 4.

2.4 Association of interactions of the *MyD88* and *TICAM1* genes with CAP in children

There was a positive multiplicative model interaction between rs7744A/G in the *MyD88* gene and rs11466711T/C in the *TICAM1* gene with a significant association with CAP susceptibility ($P<0.05$, $OR=1.420$), that is, when the genotypes of the

above two interaction sites (rs7744-rs11466711) were homozygous risk genotypes (GG-CC), the occurrence risk of CAP was significantly increased compared with homozygous wild genotype (AA-TT); there was a positive multiplicative model interaction between rs7744A/G in the *MyD88* gene and rs35299700C/T and rs35747610G/A in the *TICAM1* gene, respectively, and the severity of CAP was also significantly increased (all $P<0.05$, $OR=2.508$, 2.551), that is, when the two sites of the above interactions were homozygous risk genotypes (GG-TT, GG-AA), the risk of severe pneumonia in children with CAP was also significantly increased; There was a positive multiplicative model interaction between rs7744A/G in the *MyD88* gene and rs2292151G/A and rs35747610G/A loci in the *TICAM1* gene, respectively, which was associated with the risk of CAP complicated by sepsis (all $P<0.05$, $OR=1.451$, 2.318), that is, when the interactive genotypes of rs7744 with both rs2292151 and rs35747610 were homozygous risk genotype combinations (GG-AA), the risk of sepsis in children was significantly increased compared with all homozygous wild genotype combinations (AA-GG). See Table 5.

Table 4 Significant statistically significant association loci in association analysis of clinical characteristics

Clinical characteristic	Locus	Genetic model	Genotype	*Beta**	*SE*	Lower 95%*CI*	Upper 95%*CI*	*STAT* value	*P* value*
TICAM1									
C-reactive protein	rs6510826G/A	Recessive model	GG-GA/AA	8.060	3.900	0.417	15.700	2.067	0.039
		Codominant homozygous model	GG/AA	10.620	4.688	1.431	19.810	2.265	0.024
		Additive model	–	5.266	2.339	0.682	9.850	2.251	0.025
Sepsis	rs35747610G/A	Recessive model	GG-GA/AA	-0.429	0.183	-0.787	-0.071	-2.350	0.019
		Codominant homozygous model	GG/AA	-0.427	0.184	-0.788	-0.066	-2.318	0.021
MyD88									
Respiratory failure	rs7744A/G	Recessive model	AA-AG/GG	0.322	0.474	0.127	0.816	-2.389	0.017
		Codominant homozygous model	AA/GG	0.305	0.520	0.110	0.844	-2.286	0.022
Circulatory collapse	rs7744A/G	Recessive model	AA-AG/GG	0.238	0.568	0.078	0.724	-2.529	0.011
		Codominant homozygous model	AA/GG	0.233	0.640	0.066	0.816	-2.277	0.023

Note: * Continuous variables were analyzed using linear regression and categorical variables were analyzed using logistic regression, with adjustment for significant environmental factors such as gender and age.

Table 5 Association of the interactions of the *MyD88* and *TICAM1* gene with CAP

Interaction variable	Genotype	CAP		Severe CAP		Sepsis	
MyD88×*TICAM1*		*OR*(95%*CI*)	*P* value	*OR*(95%*CI*)	*P* value	*OR*(95%*CI*)	*P* value
rs7744×rs10422141	AA-AA/GG-TT	1.279(0.878-1.864)	0.199	0.991(0.582-1.688)	0.974	1.294(0.762-2.198)	0.340
rs7744×rs11466711	AA-TT/GG-CC	1.420(1.017-1.983)	0.039	1.430(0.865-2.364)	0.163	1.035(0.641-1.672)	0.888
rs7744×rs2292151	AA-GG/GG-AA	0.937(0.669-1.312)	0.705	1.142(0.694-1.878)	0.600	1.451(1.008-2.089)	0.045
rs7744×rs35299700	AA-CC/GG-TT	1.270(0.773-2.085)	0.345	2.508(1.273-4.941)	0.008	1.338(0.662-2.705)	0.417
rs7744×rs35747610	AA-GG/GG-AA	1.393(0.911-2.129)	0.126	2.551(1.184-5.496)	0.017	2.318(1.205-4.460)	0.012
rs7744×rs61231668	AA-TT/GG-CC	0.950(0.682-1.324)	0.763	0.941(0.577-1.535)	0.808	0.997(0.617-1.609)	0.990
rs7744×rs6510826	AA-GG/GG-AA	1.029(0.744-1.422)	0.863	0.678(0.465-1.081)	0.102	0.797(0.506-1.253)	0.325
rs7744×rs7255265	AA-CC/GG-TT	0.863(0.609-1.220)	0.403	0.885(0.536-1.461)	0.633	1.035(0.641-1.672)	0.888

Note: [CAP] community-acquired pneumonia.

3 Discussion

MyD88 is a key adaptor protein of MyD88-dependent TLRs signaling pathway. MyD88 blocking can reduce the secretion of inflammatory factors in sepsis and present a powerful protective on sepsis-induced myocardial injury[10]. Kohl et al[11] found that *Myd88*$^{-/-}$ mice could significantly reduce the expression levels of pathogen-induced inflammatory cytokines and interferon-γ-inducible genes. The rs7744 locus of the *MyD88* gene is located in the 3' UTR non-coding region, which is associated with the stability and protein expression of mRNAs. Sun et al [12] found that rs7744 polymorphism was closely associated with the severity of coronary heart disease. Jiménez-Sousa et al[13] found that GG genotypes at rs7744 were increase the risk of death in patients with septic shock. In this study, we found that the GG genotype of the *MyD88* gene at rs7744 was significantly associated with the risk of respiratory failure and circulatory failure in children with CAP. The probable mechanism may associate with activation of the MyD88-NF-κB pathway and downstream inflammatory gene expression induced by MyD88 gene. However, for the reason why the polymorphism at this locus is associated with respiratory failure and circulatory failure, rather than severity of CAP in children, need further study. TICAM1 is a key adaptor protein in TICAM1-dependent TLRs pathway, and its gene polymorphism is closely related to various diseases[14-15]. In this study, we found that the rs11466711 polymorphism of the *TICAM1* gene was associated with CAP susceptibility in children. The CC genotype at this locus may be a protective genotype for the development of CAP in children. The rs6510826 polymorphism of the gene was associated with C-reactive protein levels in children. The pulmonary inflammatory response was more intense in children carrying the AA genotype. rs11466711 and rs6510826 in the *TICAM1* gene are two non-coding SNPs located in the first intron region and are not directly involved in protein synthesis. However, intronic variants can produce cleavage variants and affect gene transcription. Moreover, the above two polymorphisms have different effects in the process of CAP in children, considering that they are related to possible alternative cleavage during transcription. Studies have shown that genetic polymorphisms involved in the TLRs pathway are closely associated with the development, progression and prognosis of sepsis[16]. In this study, we found that the AA genotype of the *TICAM1* gene at rs35747610 significantly reduced the risk of concurrent sepsis in children with CAP. The locus is a 20 kb upstream of the 5' UTR in the intergenic region. A study[17] suggested that SNPs in this region may affect the expression level of target gene miRNAs and participate in the pathophysiological mechanism of sepsis, which is closely related to the clinical manifestations and inflammatory response of patients with sepsis. Therefore, it is speculated that this association may be related to the effect of *TICAM1* gene rs35747610 polymorphism on the expression of *TICAM1* gene miRNAs and the activity of related immune pathways.

rs2292151 and rs7255265 in the *TICAM1* gene are two functional sites located on exon 2, both of which are synonymous SNPs. Although synonymous SNPs do not change the encoded amino acids, they change the affinity of the spliceosome thereby affecting cleavage. Wang et al[18] found that the rs2292151 polymorphism in the *TICAM1* gene alters the effects of *TICAM1* and is involved in the regulation of tumor necrosis factor, type I interferon synthesis, and the process in transduction and activation of innate immune signals. Cheng et al[14] analysis showed that rs4807000 and rs6510827, which are highly linked to rs7255265 in the *TICAM1* gene (both r^2=0.919), are involved in the regulation of *TICAM1* gene expression and are significantly associated with vitiligo risk in the Chinese Han population. Sahiner et al[19] study showed that rs4807000 polymorphism affects the regulation of endotoxin on IgE synthesis *in vitro* and is closely related to airway hyperresponsiveness and wheezing in asthmatic children. However, the results of this study did not show there was an association between the two exonic loci and CAP disease development. It

is mostly considered that it was related to the geographical, ethnic and disease types of the study population.

The identified genetic factors are not yet to fully explain the heritability of the disease. Studies suggest that gene-interacting and mild-effect genes are related to these "lost heritability"[20]. Numerous studies have also confirmed that gene interplay is significantly associated with disease risk[21-22]. In this study, we found that there were multiple multiplicative interaction patterns between *MyD88* and *TICAM1* genes that were significantly associated with CAP susceptibility, severity, and concurrent sepsis. Those minor loci that were not found in the association analysis of SNPs. However, actually existed could not be found in the association analysis of SNPs because their single locus variation was not enough to affect the intensity of the entire inflammatory response in the body, but these minor loci showed a strong synergistic effect in the interaction. The above results also indicate that both *MyD88* and *TICAM1* genes are involved in the immune-inflammatory regulation of CAP in children and synergistically affect the disease progression of CAP.

In summary, there are multiple functional variants in the *MyD88* and *TICAM1* genes associated with CAP in children. These variants have a synergistic effect on the disease development of CAP in children. These studies provide potential targets for individualized treatment of children with CAP and prevention of severe CAP in the future. In the clinical diagnosis and treatment practice of CAP in children, how to regulate the expression of *MyD88* and *TICAM1* genes by silencing or antagonism and coordinate the balance of different signaling pathways in the TLRs pathway is a problem to be solved and a challenge in future research.

Conflict of interest: All authors declare that no conflict of interest exists.

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