

Received: 2017.12.19  
Accepted: 2017.01.08  
Published: 2018.01.30

# Association of Single-Nucleotide Polymorphisms of C-Reactive Protein Gene with Susceptibility to Infantile Sepsis in Southern China

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ACDEFG **Chunyi Liu**  
BC **Ping Jin**  
BF **Yong Luo**  
B **Jinji Xu**  
B **Chengxiang Kong**  
B **Juan Chen**  
B **Hongbo Xie**  
B **Gang Zhou**

Department of Pediatrics, Shenzhen Baoan District Maternity and Child Care Service Centre, Shenzhen, Guangdong, P.R. China

**Corresponding Author:** Chunyi Liu, e-mail: [chunyi618@163.com](mailto:chunyi618@163.com)

**Source of support:** This study was supported by grants from the Baoan District Science and Technology Innovation Bureau Technology Research and Development Program, Shenzhen, China [grant number 2013126] and the Sanming Project of Medicine in Shenzhen [grant number SZSM201606088]

**Background:** C-reactive protein (CRP) is an important biomarker of sepsis. Several single-nucleotide polymorphisms (SNPs) in the CRP gene can determine plasma CRP levels and are risk factors in many diseases, such as cancer, arthritis, and diabetes. However, it is unknown whether polymorphisms in CRP are associated with susceptibility to and outcome of infantile sepsis. We explored the effect of these SNPs on CRP response in infantile sepsis, and compared genetic data on patients with sepsis.

**Material/Methods:** A total of 49 infants with sepsis and 20 healthy infants were enrolled during hospitalization, and 3 SNPs in the CRP gene region (rs1205, rs2808530, and rs3091244) were genotyped and then analyzed for associations with CRP levels and sepsis.





**Results:** The CRP means concentration results showed that mean CRP concentration was different in the 4 groups (healthy, sepsis, severe sepsis, and septic shock) and was positively correlated with the severity of infantile sepsis. There was also a difference in CRP SNP rs1205 between infants with septic shock and healthy infants, and between infants with septic shock and infants with sepsis. No differences were observed in SNP rs2808630 and SNP rs3091244.

**Conclusions:** Our study suggests that rs1205 genetic variability in the CRP gene determines the CRP levels in sepsis of different severities, while SNP rs3091244 and SNP rs2808630 are not associated with sepsis. However, the results of the present study on SNP rs1205, rs3091244, and rs2808630 in the CRP gene should be interpreted with caution due to limited sample size and sample heterogeneity. Large-scale, well-designed studies are needed to validate our findings.

**MeSH Keywords:** **C-Reactive Protein • Polymorphism, Single Nucleotide • Sepsis**

**Abbreviations:** **CRP** – C-reactive protein; **SNPs** – single-nucleotide polymorphisms; **ICUs** – Intensive Care Units, **ELISA** – enzyme-linked immunosorbent assay

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/908602>

 1641  6  —  37



## Background

Sepsis is a life-threatening systematic inflammatory response syndrome arising from infection [1]. A variety of infectious agents, including bacteria, viruses, fungi, and parasites, can cause sepsis [2]. Severe sepsis often results in dysfunction and in multiorgan failure, and even death [3,4]. Despite recent advances in antibiotic therapies, sepsis still is one of the major causes of mortality in intensive care units (ICUs), especially in pediatric ICUs [3]. Moreover, the incidence of sepsis in children is gradually increasing and is the leading cause of death in neonates in developed countries such as the United States and United Kingdom, and the situation is even worse in developing countries such as China [5].

The magnitude of the inflammatory response is vital in determining the consequences of sepsis. Usually, insufficient immune response to infections propagates further infection, leading to prolonged recovery time. However, the excessive inflammatory response is more detrimental because it is auto-destructive and causes tissue damage, organ injury and failure, and, eventually, death [1,3,4,6]. Genetic studies have shown that genetic variations in genes encoding host factors that are vital for controlling inflammation affect the outcome of sepsis and may be used as biomarkers to assess susceptibility to sepsis [7]. C-reactive protein (CRP) is an important biomarker of sepsis that reflects the magnitude of inflammatory response and severity of sepsis [5,8,9]. However, individual CRP values vary greatly, making it less sensitive and accurate for use in diagnosing infections at an early stage. The variable CRP values in the population are likely due to genetic variations in the CRP gene [10,11]. Genetic variations in the CRP gene have been reported to play a role in vascular damage, which may underlie the microvascular dysfunction caused by sepsis [12,13]. SNPs in the CRP gene are also associated with inflammatory processes in cardiovascular diseases and lung cancer [14,15]. However, it is unknown whether polymorphisms in the CRP gene are associated with susceptibility to and outcome of infantile sepsis. To address this, we studied the association between CRP SNPs and susceptibility to and clinical outcome of infantile sepsis in southern China.

## Material and Methods

### Subjects

In this study, we recruited 20 healthy infants and 49 infants with proven sepsis with similar age (younger than 24 months). The infants were divided into 4 groups: healthy, sepsis, severe sepsis, and septic shock. Infants were diagnosed with sepsis according to clinical criteria defined by the International Sepsis Definitions Conference in 2016 [1]. Infants with sepsis, severe

sepsis, and septic shock were classified according to international guidelines [1,4]. Parents or guardians of all the infants in this study signed informed consent, and the study was approved by the Ethics Committee of the Shenzhen Baoan District Maternity and Child Care Service Centre.

### Serum CRP measurements

Fasting blood samples were drawn from each infant and used for biochemical analysis. Serum samples were obtained from blood samples after 10 min at a centrifugation speed of 3000 rpm at 4°C and stored at -80°C until further analysis. Serum concentrations of CRP were measured by enzyme-linked immunosorbent assay (ELISA) (R&D, USA) according to the manufacturer's instructions.

### DNA sequencing and genotyping

The extraction of serum DNA used the TIANamp Blood DNA Kit method. Three common SNPs of the CRP gene (rs1205, rs2808630, and rs3091244) were genotyped in all samples. SNPs were selected as haplotype bin tagging SNPs covering the entire gene, as well as large parts of the untranslated regions of the 5' and 3' ends of the gene. The specific primers were synthesized based on the sequences issued by NCBI, including the primers for CRP rs1205: forward 5'-AGGGGACTCTTGACAGGTT-3' and reverse 5'-TTATGGACTTTGGGAGTGAGAC-3'; the primers for CRP rs2808630: forward 5'-GCTTAGAACCAACATGCCCAAAT-3' and reverse 5'-AGAAGGCCATCCCACTCCC-3'; the primers for CRP rs3091244: 5'-TGGGAGGAGCATGTTTGTTC-3' and reverse 5'-TGTTGGGGCAGGTGTGACAGAG-3'. The amplified PCR products of rs1205, rs2808630, and rs3091244 were sequenced and analyzed.

### Statistics

Statistical analysis was performed using SPSS and GraphPad Prism. The CRP levels were tested by Mann-Whitney U test and Tamhane test. The individual SNP genotypic frequency was tested by Hardy-Weinberg equilibrium. The associations between categorical variables were analyzed by the chi-square test or Fisher's exact test, as appropriate, and  $p < 0.05$  was considered significant. Continuous variables are expressed as mean  $\pm$  standard error.

## Results

We collected data from a total of 69 infants (57.9% male and 42.1% female) for this study, and their mean age was 6 months. The mean CRP levels in the 4 groups (healthy, sepsis, severe sepsis, and septic shock) of infants changed over time as the infant's condition changed. The mean CRP levels were

**Table 1.** Mann-Whitney U analysis of C-reactive protein mean concentration in four groups.

Group	Number	U mean value	SEM	95% CI	P-value
Health	20	14.75	1.42	11.77, 17.73	<0.01
Sepsis	19	32.55	4.37	23.36, 41.73	
Severe sepsis	15	39.53	2.23	34.74, 44.32	
Septic shock	14	59.96	1.67	56.36, 63.57	

SEM – standard error of mean.

**Table 2.** Tamhane test analysis of C-reactive protein levels in four groups.

Group (I)	Group (J)	Mean average value (I-J)	SD	P-value
Health	Sepsis	-17.80	4.60	<0.05
	Severe sepsis	-24.78	2.65	<0.01
	Septic shock	-45.21	2.19	<0.01
Sepsis	Severe sepsis	-6.98	4.91	0.665
	Septic shock	-27.41	4.68	<0.01
Severe sepsis	Septic shock	-20.43	2.79	<0.01

C-reactive protein values within each group were analysed by Tamhane test; SD – standard deviation.

**Table 3.** Distribution of single nucleotide polymorphism (SNP) rs1205 genotypes in 69 subjects on sepsis.

SNP	Group	CC	CT	TT	P-value
rs1205	Health (a)	3	14	3	0.069
	Sepsis (b)	6	10	4	
	Severe sepsis (c)	1	8	6	
	Septic shock (d)	2	4	8	
P-value	(a) versus (b)	0.4046	(b) versus (c)	0.6930	
	(a) versus (c)	0.2250	(b) versus (d)	0.0204*	
	(a) versus (d)	0.0274*	(c) versus (d)	0.1151	

SNP rs1205 genotypes within four group were analysed by Chi-Square test. \* P<0.05. (a) represents health group, (b) represents sepsis, (c) represents severe sepsis, (d) represents septic shock.

18.96 mg/L in the sepsis group infants admitted to a hospital, increased to a peak value of 83.06 mg/L, and decreased to 6.45 mg/L after the treatments. When severe sepsis group infants were admitted to a hospital, the mean CRP levels was 45.01 mg/L, peaked at 264.73 mg/L, and then decreased to 5.66 mg/L after the treatments. The mean CRP levels were 290 mg/L in the septic shock group patients admitted to a hospital, increased to a peak value of 689.2 mg/L, and then decreased to 5 mg/L after the treatments. There were no deaths.

The Mann-Whitney U test analysis of CRP mean concentration results showed that CRP mean concentration was different among the 4 groups (Table 1). The Tamhane multiple comparison test results indicated that CRP levels in the healthy

group were significantly different from those of the other 3 groups (Table 2). The sepsis group and severe sepsis group differed from the septic shock group, but we did not find a difference between the sepsis and severe sepsis groups (Table 2).

The distribution of SNP rs1205 genotype among the 4 groups was not significantly different (Table 3), which was also true for SNP rs2808630 (Table 4) and SNP rs3091244 (Table 5). However, it was different when the 4 groups are compared one-to-one. The results showed that there was a difference between infants with septic shock and healthy infants and between infants with septic shock and infants with sepsis (Table 3). No differences were observed in distributions of SNP rs2808630 (Table 4) and SNP rs3091244 (Table 5). Intriguingly, there were

**Table 4.** Distribution of Single nucleotide polymorphism (SNP) rs2808630 genotypes in 69 subjects on sepsis.

SNP	Group	CC	CT	TT	P-value
rs2808630	Health (a)	2	8	10	0.5398
	Sepsis (b)	3	11	5	
	Severe sepsis (c)	2	5	8	
	Septic shock (d)	2	4	9	
P-value	(a) versus (b)	0.3141	(b) versus (c)	0.2580	
	(a) versus (c)	0.9028	(b) versus (d)	0.1226	
	(a) versus (d)	0.7098	(c) versus (d)	0.7098	

SNP rs2808630 genotypes within four group were analysed by Chi-Square test. (a) represents health group, (b) represents sepsis, (c) represents severe sepsis, (d) represents septic shock.

**Table 5.** Distribution of Single nucleotide polymorphism (SNP) rs3091244 genotypes in 69 patients on sepsis.

SNP	Group	CC	CA	CT	AA	P-value
rs3091244	Health (a)	13	6	0	1	0.3016
	Sepsis (b)	14	5	0	0	
	Severe sepsis (c)	10	3	0	0	
	Septic shock (d)	12	0	0	1	
P-value	(a) versus (b)	0.5396	(b) versus (c)	0.7274		
	(a) versus (c)	0.5083	(b) versus (d)	0.7274		
	(a) versus (d)	0.1035	(c) versus (d)	0.1739		

SNP rs3091244 genotypes within four group were analysed by Chi-Square test. (a) represents health group, (b) represents sepsis, (c) represents severe sepsis, (d) represents septic shock.

**Table 6.** Chi-Square analysis of Single nucleotide polymorphism (SNP) rs1205, rs2808630, and rs3091244 in different sex.

SNP	Sex	AA	CA	CC	CT	TT	P-value
rs1205	Man	0	0	6	20	14	0.591
	Woman	0	0	6	16	7	
rs2808630	Man	0	0	3	17	20	0.893
	Woman	0	0	2	14	13	
rs3091244	Man	1	7	32	0	0	0.501
	Woman	1	8	20	0	0	

few AA genotypes in SNP rs3091244, while there were many CC genotypes (Table 5). No sex-related differences were found in the 3 SNP genotypes (Table 6).

## Discussions

CRP is an acute-phase protein produced by hepatocytes, including 29 SNPs [16]. It is reported that CRP level is related to many inflammatory reactions, cancer, heart disease, and diabetes mellitus [17,18]. Several previous studies reported the

association between CRP and risk of neonatal sepsis and pediatric sepsis. In our study, we confirmed that serum CRP levels are a high-risk factor for infantile sepsis, which is also correlated with disease severity. We also explored the association between CRP gene SNPs and infantile sepsis risk.

To the best of our knowledge, this is the first genetic association study to report on the relationship between CRP gene SNPs and infantile sepsis risk. The rs1205 SNP encodes for a functional variant of CRP and is widely reported in many diseases, including psoriasis, colorectal cancer, Takayasu arteritis,

and diabetes [19–22]. Our results showed that there was no difference between rs1205 in the CRP gene and sepsis, which is in agreement with most previous reports in other diseases. The CRP levels are correlated with the severity of sepsis, which was confirmed in our results and in previous studies [23–26]. Our results show that rs1205 levels in infants with septic shock were different from those of healthy infants and infants with sepsis, suggesting that rs1205 is related to the severity of infantile sepsis. In addition, we made the interesting observation that the percentage of TT genotype increased with the severity of infantile sepsis, while the percentage of CT genotype decreased with the severity of infantile sepsis. The genotypes changes of CRP SNP rs1205 have also been previously reported in Takayasu arteritis patients [22].

Previous studies indicated that both the alleles and genotypes of rs2808630 were significantly associated with lung cancer risk in the Chinese population [27,28]. Moreover, rs2808630 polymorphism has been shown to affect circulating CRP levels in obese patients and coronary heart disease patients [29,30], but some other studies found that rs2808630 was not related to CRP levels [31–34]. In the present study, we did not find a difference between healthy infants and infants with sepsis.

SNP rs3091244 is located in the promoter sequence of CRP gene transcription start sites [35]. Mölkänen et al. found that SNP rs3091244 was clearly associated with higher maximal CRP levels during the first week of *Staphylococcus aureus* bacteremia infections, and patients who were A-allele carriers of

SNP rs3091244 had significantly higher CRP levels compared to patients who were not carriers [36]. Abdominal aortic aneurysm patients with A or T genotype in SNP rs3091244 had higher mean CRP levels, while patients homozygous for the major C-alleles had lower mean CRP levels [37]. These results suggest that rs3091244 variation is related to CRP levels. However, our results showed that there was not a prominent variation in rs3091244 with changing CRP levels in infantile sepsis patients. Few AA genotypes and CT genotypes were found in our data, which may be because of our small sample size or due to a negative effect on sepsis.

Our study has several limitations. The small sample size limits study generalizability, and future studies should have larger sample sizes. Since CRP levels do not increase in all sepsis patients, our results may only be applicable to CRP-sensitive patients.

## Conclusions

Our study suggests that rs1205 genetic variability in the CRP gene may be a factor that determines the CRP levels in different severities of infantile sepsis. However, we found that SNP rs3091244 and SNP rs2808630 were not associated with infantile sepsis. Additionally, these findings need to be verified by future studies with larger sample sizes, especially for rs1205 and its genotypes related to sepsis severity. It may be a potential CRP SNP in infantile sepsis.

## References:

- Singer M, Deutschman CS, Seymour CW et al: The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*, 2016; 315(8): 801–10
- Balc IC, Sungurtekin H, Gurses E, Sungurtekin U, Kaptanoglu B: Usefulness of procalcitonin for diagnosis of sepsis in the intensive care unit. *Crit Care*, 2003; 7(1): 85–90
- Samransamruajit R, Uppala R, Pongsanon K et al: Clinical outcomes after utilizing surviving sepsis campaign in children with septic shock and prognostic value of initial plasma NT-proBNP. *Indian J Crit Care Med*, 2014; 18(2): 70–76
- Wu Q, Nie J, Wu FX et al: Prognostic value of high-sensitivity C-reactive protein, procalcitonin and pancreatic stone protein in pediatric sepsis. *Med Sci Monit*, 2017; 23: 1533–39
- Iwashyna TJ, Cooke CR, Wunsch H, Kahn JM: Population burden of long-term survivorship after severe sepsis in older Americans. *J Am Geriatr Soc*, 2012; 60(6): 1070–77
- Goldstein B1, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis: International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med*, 2005; 6(1): 2–8
- Hofer N, Zacharias E, Muller W, Resch B: An update on the use of C-reactive protein in early-onset neonatal sepsis: Current insights and new tasks. *Neonatology*, 2012; 102(1): 25–36
- Pepys MB, Hirschfield GM: C-reactive protein: A critical update. *J Clin Invest*, 2003; 111(12): 1805–12
- Povoa P: C-reactive protein: A valuable marker of sepsis. *Intensive Care Med*, 2002; 28(3): 235–43
- Walley KR: Biomarkers in sepsis. *Curr Infect Dis Rep*, 2013; 15(5): 413–20
- Pankow JS, Folsom AR, Cushman M et al: Familial and genetic determinants of systemic markers of inflammation: the NHLBI family heart study. *Atherosclerosis*, 2001; 154(3): 681–89
- Semeraro N, Ammolto CT, Semeraro F, Colucci M: Sepsis, thrombosis and organ dysfunction. *Thromb Res*, 2012; 129(3): 290–95
- Lange LA, Carlson CS, Hindorf LA et al: Association of polymorphisms in the CRP gene with circulating C-reactive protein levels and cardiovascular events. *JAMA*, 2006; 296(22): 2703–11
- Dupuis J, Larson MG, Vasan RS et al: Genome scan of systemic biomarkers of vascular inflammation in the Framingham Heart Study: Evidence for susceptibility loci on 1q. *Atherosclerosis*, 2005; 182(2): 307–14
- Siemes C, Visser LE, Coebergh JW et al: C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: The Rotterdam Study. *J Clin Oncol*, 2006; 24(33): 5216–22
- Danesh J, Pepys MB: C-reactive protein in healthy and in sick populations. *Eur Heart J*, 2000; 21(19): 1564–65
- Eklund CM: Proinflammatory cytokines in CRP baseline regulation. *Adv Clin Chem*, 2009; 48: 111–36
- Danesh J, Wheeler JG, Hirschfield GM et al: C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med*, 2004; 350(14): 1387–97
- Sheu WH, Wang WC, Wu KD et al: CRP-level-associated polymorphism rs1205 within the CRP gene is associated with 2-hour glucose level: The SAPPPIRe study. *Sci Rep*, 2017; 7(1): 7987
- Sudhesan A, Rajappa M, Chandrashekar L et al: Association of C-reactive protein (rs1205) gene polymorphism with susceptibility to psoriasis in South Indian Tamils. *J Clin Diagn Res*, 2016; 10(10): GC01–4

21. Fang D, Ye Y: C-reactive protein gene rs1205 polymorphism is not associated with the risk of colorectal cancer. *Biosci Rep*, 2017; 37(4): pii: BSR20170872
22. Nair AM, Goel R, Hindhumati M et al: C-reactive protein gene polymorphisms (rs1205) in Asian Indian patients with Takayasu arteritis: Associations and phenotype correlations. *Int J Rheum Dis*, 2017 [Epub ahead of print]
23. Zhang H, Wang X, Zhang Q et al: Comparison of procalcitonin and high-sensitivity C-reactive protein for the diagnosis of sepsis and septic shock in the oldest old patients. *BMC Geriatr*, 2017; 17(1): 173
24. Garnacho-Montero J, Huici-Moreno MJ, Gutierrez-Pizarra A et al: Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. *Crit Care*, 2014; 18(3): R116
25. Kim MH, Ahn JY, Song JE et al: The C-reactive protein/albumin ratio as an independent predictor of mortality in patients with severe sepsis or septic shock treated with early goal-directed therapy. *PLoS One*, 2015; 10(7): e0132109
26. Kibe S, Adams K, Barlow G: Diagnostic and prognostic biomarkers of sepsis in critical care. *J Antimicrob Chemother*, 2011; 66(Suppl. 2): ii33-40
27. Zhang S, Thakur A, Liang Y et al: Polymorphisms in C-reactive protein and Glypican-5 are associated with lung cancer risk and Gartrokine-1 influences Cisplatin-based chemotherapy response in a Chinese Han population. *Dis Markers*, 2015; 2015: 824304
28. Xu M, Zhu M, Du Y et al: Serum C-reactive protein and risk of lung cancer: A case-control study. *Med Oncol*, 2013; 30(1): 319
29. Hu M, Lee MH, Mak VW, Tomlinson B: Effect of central obesity, low high-density lipoprotein cholesterol and C-reactive protein polymorphisms on C-reactive protein levels during treatment with Rosuvastatin (10 mg Daily). *Am J Cardiol*, 2010; 106(11): 1588-93
30. Crawford DC, Sanders CL, Qin X et al: Genetic variation is associated with C-reactive protein levels in the Third National Health and Nutrition Examination Survey. *Circulation*, 2006; 114(23): 2458-65
31. Chaturvedi AK, Caporaso NE, Katki HA et al: C-reactive protein and risk of lung cancer. *J Clin Oncol*, 2010; 28(16): 2719-26
32. Veen G, Giltay EJ, van Vliet IM et al: C-reactive protein polymorphisms are associated with the cortisol awakening response in basal conditions in human subjects. *Stress*, 2011; 14(2): 128-35
33. Ottaviani S, Gorrini M, Scabini R et al: C reactive protein and alpha1-antitrypsin: Relationship between levels and gene variants. *Transl Res*, 2011; 157(6): 332-38
34. Cheng Y, Dang A, Lv N et al: Serum C-reactive protein level but not its gene polymorphism is associated with Takayasu arteritis. *Clin Rheumatol*, 2016; 35(3): 673-78
35. Xue Y, Zhang L, Fan Y et al: C-reactive protein gene contributes to the genetic susceptibility of hemorrhagic stroke in men: A case-control study in Chinese Han population. *J Mol Neurosci*, 2017; 62(3-4): 395-401
36. Molkanen T, Rostila A, Ruotsalainen E et al: Genetic polymorphism of the C-reactive protein (CRP) gene and a deep infection focus determine maximal serum CRP level in *Staphylococcus aureus* bacteremia. *Eur J Clin Microbiol Infect Dis*, 2010; 29(9): 1131-37
37. Saratzis A, Bown M, Wild B et al: C-reactive protein polymorphism rs3091244 is associated with abdominal aortic aneurysm. *J Vasc Surg*, 2014; 60(5): 1332-39