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

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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, arteritis, 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. We explored the effect of these SNPs on CRP response in infantile sepsis, and compared genetic data on patients with sepsis.

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

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Background

Sepsis is a life-threatening systemic 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′-AGGGGACCTTGGACAGGTT-3′ and reverse 5′-TTATGGACTTTGGGAGTGAGAC-3′; the primers for CRP rs2808630: forward 5′-GCTTAGACCAACATGCCCAAT-3′ and reverse 5′-AGAAGGCCCATCCACTCCC-3′; the primers for CRP rs3091244: 5′-TGGAGGAGCATGTTTGTTTTC-3′ and reverse 5′-TGTTGGGGCAGGTGTCAGAG-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 Thamhane 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 ± 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
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 were analysed by Chi-Square test. * P<0.05. (a) represents health group, (b) represents sepsis, (c) represents severe sepsis, (d) represents septic shock.

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

| Group       | Number | U mean value | SEM | 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   | <0.01   |
| Severe sepsis | 15    | 39.53        | 2.23| 34.74, 44.32   | <0.01   |
| Septic shock| 14     | 59.96        | 1.67| 56.36, 63.57   | <0.01   |

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 (I) | 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 |     |    |          |
|          | (a) versus (d) | 0.0274* |   |    |          |
|          | (b) versus (d) | 0.0204* |   |    |          |
|          | (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 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
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).

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  |         |
|         | Sepsis (b)     | 3   | 11  | 5   | 0.5398  |
|         | Severe sepsis (c) | 2   | 5   | 8   |         |
|         | Septic shock (d) | 2   | 4   | 9   |         |
| P-value | (a) versus (b) | 0.3141 |     |     |         |
|         | (a) versus (c) | 0.9028 |     |     | 0.2580  |
|         | (a) versus (d) | 0.7098 |     |     |         |
|         | (b) versus (c) |         |     |     | 0.1226  |
|         | (b) versus (d) |         |     |     |         |
|         | (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 |
|---------|----------------|-----|-----|-----|-----|---------|
| rs3901244 | Health (a)     | 13  | 6   | 0   | 1   |         |
|         | 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 |     |     |     |         |
|         | (a) versus (c) | 0.5083 |     |     |     | 0.7274  |
|         | (a) versus (d) | 0.1035 |     |     |     | 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   |         |

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.

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