Association of the ACE, GSTM1, IL-6, NOS3, and CYP1A1 polymorphisms with susceptibility of mycoplasma pneumoniae pneumonia in Chinese children

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Abstract

Mycoplasma pneumoniae is a common cause of community-acquired pneumonia (CAP) and the clinical presentation of mycoplasma pneumoniae pneumonia (MPP) varies widely. Genetic variability affecting the host response may also influence the susceptibility to MPP. Several studies have investigated the association between single nucleotide polymorphism (SNP) of some genes and the risks of CAP; however, the results were inconsistent. Here, we investigated the association of 5 functional genes and the risks of MPP, including ACE (rs4340), GSTM1 (Ins/del), IL-6 (rs1800795), NOS3 (rs1799983), and CYP1A1 (rs2606345) in a total of 715 subjects (415 cases, 300 controls) by using tetra-primer allele-specific polymerase chain reaction (PCR) and Sanger sequencing. The gene–gene interactions were analyzed using the Multifactor Dimensionality Reduction and cumulative genetic risk score approaches. Our results showed that 3 SNPs of ACE rs4340, IL-6 rs1800795, and NOS3 rs1799983 were significantly associated with the risks of MPP, while no differences were observed in genotype frequencies of GSTM1 (Ins/del) and CYP1A1 rs2606345 between both groups. The combinations of ACE rs4340D/NOS3 rs1799983T/CYP1A1 rs2606345G and ACE rs4340D/NOS3 rs1799983T contribute to the genetic susceptibility of MPP in Chinese children.

Abbreviations: BALF = bronchoalveolar lavage fluid, CAP = community-acquired pneumonia, CI = confidence intervals, HWE = Hardy–Weinberg equilibrium, MDR = Multifactor Dimensionality Reduction, MP = mycoplasma pneumoniae pneumonia, MPP = mycoplasma pneumoniae pneumonia, NPA = nasopharyngeal aspirate, OR = odds ratio, SNP = single nucleotide polymorphisms.

Keywords: case–control study, gene, mycoplasma pneumoniae pneumonia (MPP), single nucleotide polymorphisms (SNP)

1. Introduction

Mycoplasma pneumoniae (M. pneumoniae) is a type of microorganism that attaches to ciliated epithelial cells and destroys them in respiratory mucosa. It is a major cause of respiratory infections in humans of all ages worldwide, and is considered to be responsible for up to 40% of all community-acquired pneumonia (CAP) in children aged 5 to 14 years.\textsuperscript{1,2} M. pneumoniae infections occur both endemically and epidemically worldwide, with epidemic peaks every 4 to 7 years.\textsuperscript{3–5} In 2011, an epidemic of Mycoplasma pneumoniae pneumonia (MPP) was reported by several countries in Europe and Asia. In 2013, 2 typical outbreaks of M. pneumoniae infection were reported in children and adults in western Russia.\textsuperscript{6} The pneumonia caused by M. pneumoniae is often serious and can consume significant resources, and even result in great economic losses and social consequences. In recent years, more and more researches have been performed to approach the etiological factors of MPP all over the world.

Clinically, MPP exhibits an enormous variety in the severity of manifestation, from subclinical infection to septic shock. In addition, M. pneumoniae has been reported to induce not only atypical pneumonia, CAP, but also extra-pulmonary manifestations, such as autoimmune hemolytic anemia, pericarditis, myocarditis, nephritis, and meningitis with complicated pathogenesis that remains to be fully elucidated.\textsuperscript{7–9} A better understanding of what determines individual immune responses to M. pneumoniae is therefore crucial.

It has been known that different pathogens, as well as variable virulence in different strains of a pathogen/or more pathogens are the most vital factors, while host genetic factors may affect the development and progression of many infectious diseases.\textsuperscript{10} Genetic polymorphisms appear to be important in explaining variation in immune response to M. pneumoniae, and specific genes might influence susceptibility to M. pneumoniae. It is considered that genetic factors play an important role in the susceptibility to M. pneumoniae and severity of MPP.\textsuperscript{11} The relationship between genetic polymorphisms and M. pneumoniae infections has increasingly drawn public attention.

In this study, we investigated the association between genetic polymorphisms loci of 5 functional genes and the risks of MPP for all samples from the subjects of cases and controls, including
**ACE** (rs4340), **GSTM1** (Ins/del), **IL-6** (rs1800795), **NOS3** (rs1799983), and **CYP1A1** (rs2606345), as well as the gene-gene interactions using the MultiFactor Dimensionality Reduction (MDR), and cumulative genetic risk score approaches. The selection of the genes was based on the association with physiological and pathological processes probably involved in MPP, particularly in the immune and inflammatory response.

## 2. Methods

### 2.1. Subjects

The work was approved by Ethics Committee of Qilu Children’s Hospital of Shandong University. Informed written consent was obtained from the guardians of the patients. The patients’ information was anonymized before submission. All the procedures performed in the study were in accordance with the Declaration of Helsinki.

A total of 415 hospitalized children (225 boys and 190 girls, mean age 5.13 ± 2.81 years) with MPP at Qilu Children’s Hospital of Shandong University (Jinan, China) were enrolled into the study as a case group from October 2014 to March 2016. The control group consisted of 300 healthy children matched for age and gender without history of infection (154 boys and 146 girls, mean age 5.02 ± 1.63 years). All of the patients and controls were Chinese.

The children with MPP were diagnosed by experienced professionals based on criteria referenced in “Zhu Futang Practice of Pediatrics, 8th Edition.” The diagnostic criteria of *M. pneumoniae* infection are as follows: positive MP-IgM antibody (the mean titer ≥ 1:160 for acute stage) in serum and positive MP-DNA in nasopharyngeal aspirate (NPA) and/or bronchoalveolar lavage fluid (BALF).

Exclusion criteria included (1) metabolic diseases such as diabetes, hypothyroidism, and primary aldosteronism; (2) benign or malignant tumors; (3) autoimmune diseases.

### 2.2. Genotyping

Genomic DNA was obtained from peripheral blood using the TIANamp blood DNA kit (Tiangen Biotech, Beijing, China), according to the manufacturer’s instructions. Primers were designed with an online primer design tool of Primer 3, and synthesized by Invitrogen Company (Shanghai, China). The polymorphism loci of ACE rs4340, GSTM1 (Ins/del), IL-6 rs1800795, NOS3 rs1799983, and CYP1A1 rs2606345 were detected by polymerase chain reaction (PCR)-sequencing as described before. The information of primers and PCR conditions are summarized in Table 1. Allele-specific DNA products were obtained by amplification of 2-pair primers (out of 4 primers), and then sequenced on Genetic Analyzer (ABI 3730 DNA Analyzer; Applied Biosystems, Foster City, CA).

### 2.3. Statistical analysis

Statistical analysis was performed using SPSS software (version 22.0; IBM Corp. Armonk, NY). Chi-square test was utilized to assess statistical significance for genotype frequencies of the 5 single nucleotide polymorphisms (SNPs) between 2 groups and deviation from Hardy-Weinberg equilibrium (HWE). The odds ratios (ORs) and 95% confidence intervals (CIs) were used as a measure of the association between genotype frequencies and MPP occurrence, after adjusting for age and gender by logistic regression analysis. Statistical significance was accepted at *P* < .05.

SHEis software (from http://analysis.bio-x.cn/SHEisMain.htm) was used to estimate the haplotype (or allele combination) effects by maximizing the likelihood (from the observed data) that properly accounts for phase uncertainty and study design.

To observe gene-gene interaction, the MDR analysis was performed as described previously. Interaction analyses were performed in the open-source MDR software package (version 2.0). Statistical significance was determined using permutation testing in MDR *rpt* (version 1.0_beta_2), and the interaction

### Table 1

| rs no. of SNP | Primer sequences (5'-3') | PCR conditions | Amplicon length, bp |
|--------------|--------------------------|----------------|--------------------|
| CYP1A1 rs2606365 | F: ggagatggatgtttctaccatc<br>R: ctctataagggtctttcatctg | 94°C (30s) | 388 |
| | | 65.5°C (18s) | |
| IL-6 rs1800795 | F: cctgagctcaatctgtc<br>R: ccctcaagggtctttcatctg | 72°C (30s) | 155 |
| | | 34 x | |
| NOS3 rs1799983 | F: ttgcatgacttcagctttacttt<br>R: gcggcgcctcaccagactgt | 94°C (20s) | 436 |
| | | 56°C (20s) | 149 |
| | | 72°C (30s) | |
| | | 33 x | |
| GSTM1 Ins/del | F: gaactcttcggagaatcaac<br>R: gttggcctcaatctgtc | 94°C (40s) | 375 |
| | | 64.5°C (18s) | |
| | | 43°C (30s) | |
| | | 241 |
| | | 176 |
| | | 465 |
| | | 219 | |
| | | 189 | |

PCR = polymerase chain reaction, SNP = single nucleotide polymorphism.

1. PCR primer sequences are provided for amplifying products of external forward/reverse and internal allele-specific forward/reverse.
2. PCR conditions are provided as temperature (duration) of denaturation, annealing, and extension phase, respectively, followed by the number of cycles for a given SNP. Each PCR reaction was finished by a final extension phase lasting 5 min.
models were significant showing $P < .05$. The combination frequencies of genetic polymorphisms in the MPP patients ($n = 415$) were analyzed using the SAS software (version 9.1.3 portable; SAS Institute).

3. Results

3.1. Genotyping results

Genotype frequencies of ACE rs4340, NOS3 rs1799983, IL-6 rs1800795 and GSTM1 (Ins/del), and CYP1A1 rs2606345 between 2 groups are summarized in Table 2. Three genotypes (wild type, heterozygous type, and homozygous type) were found for ACE rs4340, NOS3 rs1799983, and CYP1A1 rs2606345 in the cohort, while only wild-type and heterozygous type were detected for IL-6 rs1800795 and GSTM1 (Ins/del) without homozygous type.

3.2. Genotype and allele frequency distribution

We analyzed the genotype distributions of all studied variations in the control group, and found all the genotype distributions were consistent with HWE (Table 2). Allele and genotype frequency distributions of the 5 genes in cases and controls are summarized in Table 2. The frequencies of genotype were 35.91% (D/D), 54.22% (I/D), and 9.87% (I/I) for ACE rs4340; 78.31% (G/G), 16.14% (G/T), and 5.54% (T/T) for NOS3 rs1799983; 89.16% (T/T), 10.12% (T/G), and 0.72% (G/G) for CYP1A1 rs2606345. Only 2 genotypes were found for IL-6 rs1800795 and GSTM1 (Ins/del). The genotype frequencies were 94.22% (G/G) and 5.78% (G/C) for IL-6 rs1800795; 42.41% (D/D) and 57.59% (I/*) for GSTM1 (Ins/del).

The statistical significances were found in the genotypes distributions of ACE rs4340 (42.67% vs 54.22%, OR = 1.81, 95% CI: 1.31–2.48, $P < .001$), IL-6 rs1800795 (1.33% vs 2.81%, OR = 4.44, 95% CI: 1.53–12.86) and CYP1A1 rs2606345 (1.71% vs 1.06%, OR = 2.10, 95% CI: 1.06–4.17). The genotype associated with a response in accordance with OR (protective if OR < 1, susceptible if OR > 1).

Table 2

| Genes and genotypes | Control subjects | n (%) | MPP | n (%) | Adjusted by age and sex |
|---------------------|------------------|-------|-----|-------|-------------------------|
|                     |                  |       |     |       | $P$ | OR | 95% CI |
| CYP1A1              |                  |       |     |       |     |     |       |
| rs260345            |                  |       |     |       |     |     |       |
| T                   | 563              | 93.83 | 782 | 94.22 | .76 | 0.93 | 0.60–1.45 |
| G                   | 37               | 6.17  | 48  | 5.78  | .52 | 0.86 | 0.53–1.38 |
| T/T                 | 26 (26.41)       | 88    | 370 (368.39) | 89.16 | .52 | 0.86 | 0.53–1.38 |
| T/G                 | 35 (34.72)       | 11.67 | 42 (45.220 | 10.12 | .52 | 0.86 | 0.53–1.38 |
| G/G                 | 1 (1.14)         | 0.33  | 3 (1.39) | 0.72  | .5  | 2.14 | 0.22–20.69 |
| T/G+G/G             | 36               | 45    | 45  | 45    | .63 | 0.89 | 0.56–1.42 |
| GSTM1               |                  |       |     |       |     |     |       |
| Del (D/D)           |                  |       |     |       |     |     |       |
| HWP                 | NA               |       |     |       |     |     |       |
| Ins (I/*)           |                  |       |     |       |     |     |       |
| D/D                 | 125 (150.52)     | 41.67 | 176 (210.41) | 42.41 | .84 | 0.97 | 0.71–1.31 |
| I/*                 | 175 (123.96)     | 58.33 | 239 (307.18) | 57.59 | .84 | 0.97 | 0.71–1.31 |
| IL-6                |                  |       |     |       |     |     |       |
| rs180079            |                  |       |     |       |     |     |       |
| G                   | 596              | 99.33 | 806 | 97.11 | .003 | 4.44 | 1.53–12.86 |
| C                   | 4                | 0.67  | 24  | 2.81  | .71  | 0.93 | 0.63–1.37 |
| G/G                 | 296 (296.01)     | 98.67 | 391 (391.35) | 94.22 | .003 | 4.54 | 1.56–13.23 |
| G/C                 | 4 (3.97)         | 1.33  | 24 (23.31) | 5.78  | .003 | 4.54 | 1.56–13.23 |
| C/C                 | 0 (0.01)         | 0     | 0 (0.35) | 0     | 1    | 0.76 | 0.015–13.23 |
| G/C+C/C             | 4                | 24    | 24  | 24    | .003 | 4.54 | 1.56–13.23 |
| NOS3                |                  |       |     |       |     |     |       |
| rs1799983           |                  |       |     |       |     |     |       |
| Asp298Glu           |                  |       |     |       |     |     |       |
| G                   | 538              | 80.67 | 709 | 85.42 | .003 | 4.44 | 1.53–12.86 |
| C                   | 162              | 19.33 | 191 | 14.58 | .003 | 4.44 | 1.53–12.86 |
| 32                  | 158              | 0.018 | 1.48 | 1.07–2.05 | .71  | 0.93 | 0.63–1.37 |
| G/G                 | 241 (241.20)     | 80.33 | 320 (320.22) | 78.31 | .71  | 0.93 | 0.63–1.37 |
| G/T                 | 56 (55.59)       | 18.67 | 69 (103.36) | 16.14 | .001 | 6.53 | 1.95–21.87 |
| G/T+T/T             | 59               | 85    | 600 | .3    | 1.21 | 0.84–1.75 |
| ACE                 |                  |       |     |       |     |     |       |
| rs4340              |                  |       |     |       |     |     |       |
| Alu-287bp           |                  |       |     |       |     |     |       |
| G                   | 406              | 67.67 | 509 | 61.33 | .014 | 1.32 | 1.06–1.65 |
| T                   | 194              | 32.33 | 321 | 38.67 | .014 | 1.32 | 1.06–1.65 |
| D/D                 | 13 (13.37)       | 46.33 | 139 (156.07) | 35.91 | .014 | 1.32 | 1.06–1.65 |
| I/*                 | 12 (13.37)       | 42.67 | 231 (196.89) | 54.22 | .014 | 1.32 | 1.06–1.65 |
| N                   | 33 (31.36)       | 11    | 45 (62.07) | 9.87  | .23  | 1.36 | 0.82–2.26 |
| N+I                 | 161              | 276   |     |       | <.001 | 1.71 | 1.26–2.33 |

CI = confidence interval, HWP = Hardy–Weinberg probability, N/A = not applicable, OR = odds ratio.

Genotype associated with a response in accordance with OR (protective if OR < 1, susceptible if OR > 1).

* Significant $P < .05$. 

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5.78%, OR = 4.54, 95% CI: 1.56–13.23, \(P = .003\) as well as allele distribution of ACE rs4340 I (OR = 1.32, 95% CI: 1.06–1.65, \(P = .014\)) and NO3 rs179983 T (OR = 6.53, 95% CI: 1.95–21.87, \(P = .001\)). IL-6 rs1800795 C (OR = 4.44, 95% CI: 1.53–12.86, \(P = .003\)) between the case and control groups (Table 2), indicating that ACE rs4340 I/D, IL-6 rs1800795 G/C, and NO3 rs179983 G/T were associated with increased risks of MPP, although no significant differences in genotype or allele frequencies were found for GSTM1 (Ins/del) and CYP1A1 rs2606345 G/T between the 2 groups (Table 2).

3.3. Gene–gene interaction analysis

We constructed possible allele combinations of ACE rs4340, GSTM1 (Ins/del), IL-6 rs1800795, NO3 rs179983, and CYP1A1 rs2606345 to analyze gene–gene interactions (Table 3). A 3-fold increased risk was identified in the combinations of ACE rs4340/D, NO3 rs179983/C, and CYP1A1 rs2606345 G/T (OR = 3.316, 95% CI: 1.879–5.851). The combination frequencies of ACE rs4340 and NO3 rs179983 genetic polymorphisms in the MPP patients (n = 415) were also analyzed and showed significant differences between the cases and controls (OR = 3.316, 95% CI: 1.879–5.851).

4. Discussion

In this study, we investigated whether the ACE rs4340 I/D, GSTM1 (Ins/del), IL-6 rs1800795 G/C, CYP1A1 rs2606345 G/T, and NO3 rs179983 G/T polymorphisms were associated with the risks of MPP in Chinese children. Our data suggested that ACE rs4340 genotype was related to increased risks of MPP, which is consistent with the findings of Salnikova et al.\(^{[13]}\)

ACE gene, encoding human angiotensin-converting enzyme, located on chromosome 17q23 spans over 24 kb in length and contains 26 exons. A meta-analysis revealed a causal association between ACE rs4340 polymorphism at 16th intron and pneumonia risk. The ACE rs4340 SNP could suppress cough reflex by decreasing bradykinin and substance P levels, while increasing the probability of developing pneumonia.\(^{[13]}\) The plasma ACE levels were partly regulated by I/D polymorphism of ACE gene in human, with DD homozygotes having roughly twice levels than II homozygotes.\(^{[16]}\) However, previous epidemiological studies reported different conclusions regarding the role of rs4340 SNP and pneumonia predisposition. Caldeira et al.\(^{[17]}\) did a systematically longitudinal study to access the association of ACE inhibitors and risk of pneumonia. They found ACE inhibitors not only played a protected role in risk of pneumonia but were also associated with reduced mortality related to pneumonia. Li et al.\(^{[18]}\) performed a meta-analysis of ACE I/D polymorphism in ACE inhibitor related cough and discovered that ACE I/D polymorphism was a genetic, age-dependent risk factor for ACE inhibitor related cough. A study of Russian population indicated that the ACE DD genotype was related to

| Table 3 |
| --- |
| Allele combinations of ACE, GSTM1, IL6, NOS, and CYP polymorphisms with synergistic effects by MDR method. |
| Characteristics | Case (n = 415) | Control (n = 300) | Chi-square | Fisher’s \(P\) | Odds ratio [95% CI] |
| --- |
| ACE, GSTM1, IL6, NOS, CYP | | | | | |
| D CG G G* | 86.45 (0.104) | 1.00 (0.002) | 65.961 | 8.33e-015 | 72.555 [10.074–522.562] |
| D I G G* | 138.52 (0.167) | 0.25 (0.000) | 113.559 | 4.66e-015 | 512.169 [52.571–4969.803] |
| D I G G* | 48.3 (0.006) | 91.57 (0.153) | 117.008 | 2.00e-015 | 0.033 [0.013–0.083] |
| I D C G* | 183.04 (0.221) | 1.00 (0.002) | 155.462 | 1.55e-015 | 180.512 [25.206–1292.721] |
| I D G T* | 46.2 (0.006) | 132.67 (0.221) | 183.612 | 2.22e-015 | 0.020 [0.008–0.050] |
| I D G T* | 1.27 (0.002) | 21.46 (0.036) | 25.421 | 4.77e-007 | 0.042 [0.007–0.254] |
| I I C G* | 249.01 (0.300) | 1.75 (0.003) | 223.875 | 0.00e+00 | 158.888 [35.791–705.351] |
| I I G G* | 4.90 (0.006) | 205.18 (0.342) | 311.676 | 0.00e+00 | 0.011 [0.004–0.028] |
| I I G T* | 0.06 (0.000) | 18.31 (0.031) | 23.899 | 1.05e-006 | 0.002 [0.000–0.032] |
| ACE, IL6, NOS, CYP | | | | | |
| D C G G* | 22.69 (0.273) | 1.57 (0.003) | 193.860 | 3.44e-015 | 147.700 [30.671–711.268] |
| D G G* | 42.19 (0.517) | 2.43 (0.004) | 450.558 | 1.33e-015 | 286.179 [80.464–1018.055] |
| I C G G* | 10.63 (0.013) | 338.65 (0.504) | 582.107 | 2.22e-016 | 0.009 [0.005–0.017] |
| I G G* | 3.00 (0.004) | 20.35 (0.034) | 19.767 | 8.94e-006 | 0.104 [0.031–0.351] |
| I G T G* | 1.15 (0.001) | 40.02 (0.007) | 51.87 | 0.09e-013 | 0.91 [0.032–0.125] |
| ACE, NOS, CYP | | | | | |
| D G* | 229.98 (0.277) | 164.55 (0.274) | 0.020 | 0.886365 | 1.017 [0.803–1.289] |
| D G* | 69.56 (0.064) | 17.39 (0.039) | 18.436 | 1.79e-005 | 3.076 [1.798–5.263] |
| I G* | 440.58 (0.531) | 341.02 (0.568) | 1.932 | 0.164580 | 0.858 [0.692–1.065] |
| I G* | 23.33 (0.028) | 20.37 (0.034) | 0.393 | 0.530647 | 0.824 [0.451–1.509] |
| I G* | 41.88 (0.050) | 40.04 (0.067) | 1.684 | 0.194944 | 0.745 [0.476–1.164] |
| ACE, NOS | | | | | |
| D G* | 245.08 (0.295) | 176.96 (0.295) | 0.000 | 0.988729 | 1.002 [0.796–1.261] |
| D G* | 75.92 (0.091) | 17.04 (0.028) | 22.789 | 1.86e-006 | 3.444 [2.014–5.888] |
| I G* | 463.92 (0.559) | 361.04 (0.602) | 2.613 | 0.106061 | 0.839 [0.678–1.038] |
| I G* | 45.08 (0.054) | 44.96 (0.075) | 2.510 | 0.113222 | 0.709 [0.463–1.087] |

Global result: Total control = 600.0, total case = 830.0.
Global chi-square is 1202.664971, while df = 11 (frequency < 0.03 in both control and case has been dropped).
ACE = angiotensin-converting enzyme, CI = confidence interval, NOS = nitric oxide synthase.
the increased risk of CAP and nosocomial pneumonia (NP),[13] while Van de Garde reported that the ACE I/D polymorphism had no effect on the susceptibility and outcome of pneumonia in the Dutch white population.[19] In this study, we detected the protective factor of ACE insertion in MPP-control study. Given that ID heterozygous has a higher risk than the carriers of DD and II genotypes, the ACE I/D polymorphism can also predispose to MPP. Our result indicated the marked ethnic differences between polymorphisms of the ACE gene with the higher prevalence of the DD genotype in Chinese children than that of Russian population.[18]

Although no differences were found for GT genotype of NOS3 rs1799983 between patients and controls, the prevalence of TT genotype was significantly higher in the cases than in the controls (Table 2). Nitric oxide (NO) is an important mediator in regulation cardiovascular homeostasis. NO is synthesized from L-arginine and oxygen by 4 major isofoms of NO synthase (NOS): neuronal NOS, endothelial NOS (eNOS), inducible NOS, and mitochondrial NOS. There are 3 NOS genes (NOS1, NOS2, and NOS3) in human and the NOS3 gene has been considered to play a more important role than others for respiratory problems because human airway epithelial cells express only NOS3. However, to date, there is limited research on the relationship between NOS3 polymorphisms and lung function in humans, and the results were inconsistent.[20][21] The NOS3 gene, located at 7q35 to 7q36 region of chromosome 7 in humans, encodes NOS3 and a number of genetic variations have been observed. However, to date, there is little information on the relationship between NOS3 polymorphisms and lung function in humans and the available results are inconsistent.[22]

Several studies demonstrated that some NOS3 polymorphisms had functional effects on NO formation and were associated with a wide range of disease susceptibility and drug responses.[23][24] The G894T polymorphism (rs1799983), which is located in exon 7 of the gene resulting in the amino acid change (Glu298Asp), had been reported to be associated with reduced basal NO production.[25] The variant Asp allele for Glu298Asp polymorphism reduces NOS3 transcriptional activity and NO production in the endothelial cells.[26]

In our study, we found that the interleukin-6 (IL-6) genotypes were significantly associated with MPP. Children carrying IL-6 rs1800795 G/G, G/C, or C/C genotypes were susceptible to MPP. IL-6 is a pleiotropic cytokine involved in many physiological and pathological processes, particularly in the inflammatory response.[27] Conflicting results have been reported about the function of IL-6 genetic variability, especially the IL-6 rs1800795 G/C SNP. Martín-Loeches et al.[28] studied the genetic variability of IL-6 in white Spanish patients with CAP and pneumococcal CAP (P-CAP). The results of this study were that IL-6 rs1800795 G allele played an important protective role against the severity and outcome of P-CAP. By contrast, no effect on severity was observed in 2 other studies in critically ill patients with sepsis.[29]

Our results confirmed that the frequency of IL-6 rs1800795 G/C heterozygous was significantly higher in patients with MPP than in healthy controls. The IL-6 rs1800795 G/C polymorphism carriers had a higher susceptibility to MPP (OR 4.54, 95% CI: 1.56–13.231, P = .0025). Interestingly, only wild type and heterozygous of IL-6 rs1800795 were found in the cohort, and the distribution of IL-6 genotype differed from other populations. Molecular heterosis might be a reason, as it is rather common in humans.[30]

In order to evaluate the diagnostic prediction of MPP mediator genes polymorphisms, the risk polymorphisms of ACE rs4340 and NOS3 rs1799983 were combined and analyzed using SAS software, and the combination frequencies were found significant differences between the cases and the controls (OR = 3.316, 95% CI: 1.879–5.851). So, a new OR combining ACE rs4340 and NOS3 rs1799983 genetic risk factors was generated, which could detect the patients with the greatest risk of suffering a future MPP. Future studies are necessary to verify the observed associations.

Ethnic background is known to influence polymorphism frequencies and their effects on the disease. In this study, the GSTM1 (Ins/del) and CYP1A1 rs2606345T/G polymorphisms were not associated with risks of MPP in the Chinese children, which was different from the report in Russian population.[13]

In conclusion, our data demonstrated that 3 SNPs of ACE rs4340, IL-6 rs1800795, and NOS3 rs1799983 were significantly associated with the risks of MPP; moreover, the combinations of ACE rs4340D/ NOS3rs1799983T/CYP1A1 rs2606345G and ACE rs4340D/ NOS3 rs1799983T contribute to the genetic susceptibility of MPP in Chinese children. However, we only investigated 5 genes’ SNPs for the risks of MPP with limited samples. Further investigation will be needed for the relation between SNPs and MPP.

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