

Article

Association of CFH Polymorphism with Susceptibility to Sepsis Caused by *Pseudomonas Aeruginosa* in Chinese Han Populations: A Multi-center Study

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Abstract: Complement factor H (CFH) serves as a major down-regulator in the complement system, often utilized by bacterial pathogens to evade complement attack. Yet, little is currently known about the genetic correlation of CFH polymorphisms with sepsis due to various microbial infections. A case-control method (488 septic patients and 527 healthy individuals) was carried out in this study to investigate the genetic relationship between CFH polymorphisms (rs3753394 C/T, rs1065489 G/T and rs1061170 C/T) and susceptibility to sepsis caused by bacterial infections in Chinese Han populations. Our findings indicated that the frequency of rs3753394 CT/TT genotype in the septic patients with *P. aeruginosa* was significantly higher than that in the control individuals ($P=0.033$, $OR=2.668$, $95\%CI=1.072-6.334$). The rs3753394 T allele frequency in the *P. aeruginosa*-infected patients was significantly increased, compared to that in the healthy controls ($P=0.014$, $OR=1.68$, $95\%CI=1.118-2.538$). Moreover, these significant differences of rs3753394 genotype and allele frequencies remained after multiple testing corrections [P (corr.) = 0.033 for genotype; P (corr.) = 0.033 for allele]. The current study highlighted the significance of CFH polymorphism rs3753394 as a potential biomarker for targeting *P. aeruginosa* infection in critically ill patients.

Keywords: Sepsis; CFH; Single nucleotide polymorphisms; *Pseudomonas Aeruginosa*

1. Introduction

Sepsis, a major cause of death worldwide, is recognized as a syndrome of host organ dysfunction due to microbial infection [1]. Normally, the innate immune dysregulation gets involved in the pathogenesis of sepsis [2, 3]. Numerous studies further indicated that genetic variability in genes regarding the innate immune response was shown in functional correlation with predisposition to and prognosis of sepsis in critically ill patients [4-7].

Host complement system occupies pivotal roles in relation to the innate immune response for defense against bacterial invasion, whereas the excessive complement cascade may produce substantial adverse effects on the host cells, thereby accelerating the progression of sepsis [8-10]. Complement factor H (CFH) acts as a major down-regulator in the complement system to prevent damage to self, primarily via the inactivation of complement component C3b, following the repression of complement alternative pathway (AP) [11, 12]. Any altered expression or function of CFH induced by genetic variations in CFH gene has the most decisive influence on the immune and inflammatory conditions in human immunological diseases [13-16]. Moreover, these mutations in CFH gene may also affect genetic mechanisms that underlie susceptibility or resistance to pathogenic bacterial infections [17-20], highly suggestive of the relevance of CFH polymorphisms in gene to sepsis.

Three functional single nucleotide polymorphisms (SNPs) in CFH gene, including rs3753394 C/T, rs1065489 G/T and rs1061170 C/T, were reported to alter the expression levels or functions of human CFH, thus contributing to genetic proneness to various diseases [21-25]. The SNP rs3753394 C/T in the promoter region of CFH gene lies in a nuclear factor-kappa B (NF- κ B) responsive element and may participate in the modulation of CFH transcription [21]. This SNP could up-regulate CFH levels and decrease the risk of developing severe dengue [23]. The rs1065489 G/T polymorphism in the short consensus repeat (SCR)-16 of CFH produces a Glu963Asp change and may impact on C3b binding affinity, resulting in the inactivation of AP [14, 21]. This polymorphism could elevate the susceptibility to anterior uveitis and haemolytic uraemic syndrome, but reduce the risk of meningococcal infection [14, 22, 24]. The rs1061170 C/T in the SCR-7 of CFH may influence CFH combining with heparin or C-reactive protein by substituting tyrosine with histidine, strongly associated with predisposition to age-related macular degeneration [25]. This genetic variant also revealed the low risk of erysipelas and recurrent tonsillitis via impairing CFH binding onto *group A streptococcus* [20]. Yet, little is currently known about the genetic correlation of CFH polymorphisms with various pathogenic causes of sepsis.

In this study, we performed a case-control method to identify the genetic relationship between CFH SNPs (rs3753394 C/T, rs1065489 G/T and rs1061170 C/T) and susceptibility to sepsis caused by bacterial infections in Chinese Han populations. We recruited a total number of 1015

subjects (488 septic patients and 527 healthy individuals) from three independent centers across China in this study cohort.

2. Materials and Methods

2.1 Ethics Declaration

The Ethics Committees of the Central Hospital of Wuhan (Hubei, China), the Affiliated Hospital of Guangdong Medical University (Guangdong, China) and the Second Affiliated Hospital of Harbin Medical University (Heilongjiang, China) approved this study which followed the Declaration of Helsinki. All participants or their family members signed the written informed consent before their recruitment in this study.

2.2 Study Population

A total number of 488 Chinese Han septic patients were recruited from three independent Intensive Care Units (ICUs) nationwide between 2015 and 2018. All patients were diagnosed as sepsis or septic shock in accordance with The Third International Consensus Definitions [1]. Any septic patient was excluded, when under 16 years old and suffering from malignancies, diabetes mellitus, autoimmune diseases, human immunodeficiency virus infection, acquired immune deficiency syndrome and any other immunosuppressive or immune-dysregulated causes (such as steroid and radiation therapy). Then clinical data were collected for every enrolled septic patients, including age, gender, organ dysfunctions, infection origins, infection types, pathogenic bacteria, Acute Physiology and Chronic Health Evaluation (APACHE)-II and Sequential Organ Failure Assessment (SOFA) scores, administration days in ICUs and laboratory values [except 66.60% cases for Lactate; 81.35% cases for Procalcitonin]. Meanwhile, a total number of 527 Chinese Han healthy individuals were also recruited from each Health Examination Center of the above three hospitals. No healthy individuals suffered from any acute disease within the latest three months, cancer, hypertension, autoimmune diseases, and major cardiac, pulmonary, renal, hepatic or endocrinological diseases.

2.3 Blood Sample Collection

Whole blood samples were collected from enrolled healthy individuals and patients within 12 hours upon diagnosing sepsis or septic shock, which were preserved in the tubes containing ethylene diamine tetraacetic acid. After separating plasma, samples were well stored at -80°C.

2.4 DNA Extraction

In line with the protocol of TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China), genomic DNA samples were isolated and kept at -80°C for subsequent genotyping. In addition, DNA quality was verified via the absorbance at wavelengths of 260 and 280 nm measured by Epoch microplate spectrophotometer (BioTek, USA). The 260/280 ratio between 1.8 and 2.0 indicated that DNA purity was validated.

2.5 Genotyping

Three SNPs in CFH gene (rs3753394 C/T, rs1065489 G/T and rs1061170 C/T) were genotyped for 1015 subjects by harnessing ABI PRISM SNaPshot technique (Applied Biosystems, Carlsbad, CA, USA). Polymerase chain reaction was used for genotyping. Then, ten percent of the samples were selected randomly to re-genotype for independent validation. GeneMapper 4.1 (Applied Biosystems, Carlsbad, CA, USA) was utilized for analyzing the final data.

2.6 Statistical Analyses

SPSS version 25.0 (IBM, NY, USA) was used to perform statistical analyses. The differences of demographic and clinical characteristics between septic patients and healthy controls were measured by student's t-test or chi-squared test. Logistic regression analysis adjusting for age and gender was carried out to assess the correlation between sepsis and CFH polymorphisms and determine odds ratios (ORs) and 95% confidence intervals (CIs). Chi-squared test or fisher's exact test was used for calculating the genotype and allele frequencies of septic patients and healthy controls. The association of CFH SNPs with disease severity was tested by one-way ANOVA. Hardy-Weinberg equilibrium (HWE) was used to evaluate the deviation of genotype or allele frequency. Power analysis was conducted by QUANTO 1.2 software (University of Southern California, LA, USA). Benjamin-Hochberg (BH) corrections were utilized to analyze the false discovery rate for multiple hypotheses testing. All data were summarized as percentage frequencies or mean \pm SEM. The partial missing data were assumed not influenced because of categorical variables and the median of continuous variables. P value < 0.05 was identified as statistical significance.

3. Results

3.1 Demographic and Clinical Parameters

Demographic and clinical characteristics of all 1015 subjects were listed in Table 1. This study cohort was composed of 527 healthy controls, 242 sepsis and 246 septic shock patients. The distributions of age and gender between cases and controls were significantly different. Accordingly, logistic regression with controlling age and gender was performed for the subsequent analyses. Respiratory tract was the most common origin of infection (76.23%), followed by abdomen (19.47%), urinary tract (15.78%) and primary bloodstream (14.96%). Gram-negative infection (35.45%) was the major infective type, while gram-positive infection only occupied 6.35%. *Acinetobacter baumannii* (22.95%), *Pseudomonas aeruginosa* (11.27%), *Escherichia coli* (11.07%) and *Staphylococcus* (8.20%) were the main pathogenic bacteria detected in this study. The average APACHE-II and SOFA scores were up to 19.44 \pm 6.40 and 7.85 \pm 4.18, respectively.

Table 1. Demographic and clinical characteristics of all patients with sepsis and healthy controls.

Characteristics	All patients (n=488)	Controls (n=527)
Age (years, mean \pm SEM)	62.17 \pm 17.43*	42.93 \pm 13.33

Male/Female, n	313/175*	278/249
Organ dysfunction, n (%)		
One	160 (32.79%)	N.A
Two or above	328 (67.21%)	N.A
Sepsis status, n (%)		
Sepsis	242 (49.59%)	N.A
Septic Shock	246 (50.41%)	N.A
Origin of infection, n (%)		
Respiratory tract infection	372 (76.23%)	N.A
Abdominal infection	95 (19.47%)	N.A
Urinary tract infection	77 (15.78%)	N.A
Primary bloodstream infection	73 (14.96%)	N.A
Intracranial infection	8 (1.64%)	N.A
Catheter associated infection	7 (1.43%)	N.A
Wound infection	6 (1.23%)	N.A
Others	18 (3.69%)	N.A
Type of infection, n (%)		
Gram-negative	173 (35.45%)	N.A
Gram-positive	31 (6.35%)	N.A
Mixed Gram-negative and -positive	37 (7.58%)	N.A
Fungus	43 (8.81%)	N.A
Pathogenic bacteria, n (%)		
Acinetobacter baumannii	112 (22.95%)	N.A
Klebsiella pneumoniae	37 (7.58%)	N.A
Pseudomonas aeruginosa	55 (11.27%)	N.A
Escherichia coli	54 (11.07%)	N.A
Enterococcus	12 (2.46%)	N.A
Staphylococcus	40 (8.20%)	N.A
Streptococcus	11 (2.25%)	N.A
Aspergillus	10 (2.05%)	N.A
Candida	37 (7.58%)	N.A
Others	57 (11.68%)	N.A
APACHE II score (Day 1), mean±SEM	19.44±6.40	N.A
SOFA score (Day 1), mean±SEM	7.85±4.18	N.A
Average hospitalized days in ICU, mean±SEM	10.76±10.29	N.A
Lactate (mmol/L, mean±SEM) [†]	3.62±3.57	N.A
WBC (10 ⁹ /L, mean±SEM)	17.74±10.09	N.A
PCT (ng/ml, mean±SEM) [†]	24.08±43.85	N.A

APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; ICU, Intensive Care Unit; WBC, White Blood Cell Count; PCT, Procalcitonin; N.A, not applicable

*P<0.001 compared to healthy control group.

†Partial data missing: included 66.60% cases for Lactate; 81.35% cases for PCT.

3.2 Genotype and Allele Frequencies among Different Sepsis Status and Controls

In this study, 527 controls, 240 sepsis and 244 septic shock cases were, in the aggregate, successfully genotyped for CFH SNP rs3753394 C/T, rs1065489 G/T and rs1061170 C/T. The genotype and allele frequency distributions of the three CFH SNPs in sepsis subtypes and controls were shown in Table 2. The detected frequency distributions had the consistency of HWE (all P values > 0.05). Power analysis based on the sample size indicated 99.50% power for rs3753394 C/T, 99.50% power for rs1065489 G/T and 45.60% power for rs1061170 C/T to achieve a significance level of 0.05 with an OR of 1.5. The genotype and allele frequencies of rs3753394 C/T, rs1065489 G/T and rs1061170 C/T did not differ among sepsis subtypes and controls. As summarized in Table 3, no statistical significance in differences of CFH polymorphism genotype and allele frequencies between sepsis and septic shock subtypes was observed as well.

Table 2. Genotype and allele frequency distributions of CFH polymorphisms between sepsis subtypes and healthy controls.

Genotype	Controls n (%)	Sepsis n (%)	P ₁ value	OR (95% CI)	Septic shock n (%)	P ₂ value	OR (95% CI)
rs3753394 C/T							
Total	527	240			244		
CC	111 (21.06%)	46 (19.17%)		1	51 (20.90%)		1
CT	252 (47.82%)	116 (48.33%)	0.715	1.10 (0.67, 1.78)	113 (46.31%)	0.644	1.12 (0.70, 1.79)
TT	164 (31.12%)	78 (32.50%)	0.546	1.17 (0.70, 1.96)	80 (32.79%)	0.622	1.14 (0.69, 1.87)
Allele							
C	474 (44.97%)	208 (43.33%)		1	215 (44.06%)		1
T	580 (55.03%)	272 (56.67%)	0.549	1.07 (0.86, 1.33)	273 (55.94%)	0.737	1.04 (0.84, 1.29)
rs1065489 G/T							
Total	527	240			244		
GG	128 (24.29%)	55 (22.92%)		1	59 (24.18%)		1
GT	251 (47.63%)	122 (50.83%)	0.695	1.10 (0.70, 1.73)	110 (45.08%)	0.651	1.11 (0.71, 1.75)
TT	148 (28.08%)	63 (26.25%)	0.806	1.07 (0.64, 1.77)	75 (30.74%)	0.216	1.37 (0.83, 2.24)
Allele							
G	507 (48.10%)	232 (48.33%)		1	228 (46.72%)		1
T	547 (51.90%)	248 (51.67%)	0.933	0.99 (0.80, 1.23)	260 (53.28%)	0.614	1.06 (0.85, 2.62)

rs1061170 C/T							
Total	527	240			244		
CC	1 (0.19%)	1 (0.42%)		1	1 (0.41%)		1
CT	54 (10.25%)	27 (11.25%)	0.678	0.51 (0.02, 12.32)	23 (9.43%)	0.674	0.51 (0.02, 12.03)
TT	472 (89.56%)	212 (88.33%)	0.711	0.55 (0.02, 12.80)	220 (90.16%)	0.720	0.56 (0.03, 12.84)
Allele							
C	56 (5.31%)	29 (6.04%)		1	25 (5.12%)		1
T	998 (94.69%)	451 (93.96%)	0.563	0.87 (0.56, 1.37)	463 (94.88%)	0.876	1.04 (0.64, 1.67)

OR: odds ratio; 95% CI: 95% confidence interval.

P₁: healthy control group versus sepsis group; P₂: healthy control group versus septic shock group.

Table 3. Genotype and allele frequency distributions of CFH polymorphisms in sepsis subtypes.

Genotype	Sepsis	Septic shock	OR	
	n (%)	n (%)	P value	(95% CI)
rs3753394 C/T				
Total	240	244		
CC	46 (19.17%)	51 (20.90%)		1
CT	116 (48.33%)	113 (46.31%)	0.540	0.86 (0.54, 1.39)
TT	78 (32.50%)	80 (32.79%)	0.716	0.91 (0.55, 1.51)
Allele				
C	208 (43.33%)	215 (44.06%)		1
T	272 (56.67%)	273 (55.94%)	0.820	0.97 (0.76, 1.25)
rs1065489 G/T				
Total	240	244		
GG	55 (22.92%)	59 (24.18%)		1
GT	122 (50.83%)	110 (45.08%)	0.409	0.83 (0.53, 1.30)
TT	63 (26.25%)	75 (30.74%)	0.705	1.10 (0.67, 1.81)
Allele				
G	232 (48.33%)	228 (46.72%)		1
T	248 (51.67%)	260 (53.28%)	0.616	1.07 (0.83, 1.37)
rs1061170 C/T				
Total	240	244		
CC	1 (0.42%)	1 (0.41%)		1
CT	27 (11.25%)	23 (9.43%)	0.906	0.84 (0.05, 14.40)
TT	212 (88.33%)	220 (90.16%)	0.984	1.03 (0.06, 16.76)
Allele				
C	29 (6.04%)	25 (5.12%)		1
T	451 (93.96%)	463 (94.88%)	0.534	1.19 (0.68, 2.03)

OR: odds ratio; 95% CI: 95% confidence interval.

3.3 Genotype and Allele Frequencies among Different Types of Infection and Controls

Considering CFH SNPs had influences on genetic mechanisms involving pathogenic bacterial infections [17-20], the relevance of CFH polymorphisms to different pathogens was further analyzed in this study. Yet, in virtue of the insufficient presence of rs1061170 C allele in septic cases and healthy controls for any meaningful analysis to be done, genotype and allele frequency distributions of only rs3753394 C/T and rs1065489 G/T in Gram-negative, Gram-positive, mixed G - and G +, fungus and controls were presented in Table 4. No significant difference was found in the genotype and allele frequencies of the two CFH SNPs among four infection types and healthy controls. Then, the distributions of CFH polymorphism rs3753394 C/T and rs1065489 G/T genotype and allele frequencies in control group, major Gram-negative and Gram-positive bacteria were described in Table 5 and 6, respectively. Among four Gram-negative bacteria identified in this study, there were statistical significance in differences of rs3753394 genotype and allele frequencies between *Pseudomonas aeruginosa* cases and controls. The frequency of rs3753394 CT/TT genotype in the septic patients with *P. aeruginosa* was significantly higher than that in the control individuals ($P_3=0.033$, OR=2.668, 95%CI=1.072-6.334). The rs3753394 T allele frequency in the *P. aeruginosa*-infected patients was significantly increased, compared to that in the healthy controls ($P_3=0.014$, OR=1.68, 95%CI=1.118-2.538). Moreover, these significant differences of rs3753394 genotype and allele frequencies remained after BH corrections [P_3 (corr.) = 0.033 for genotype; P_3 (corr.) = 0.033 for allele]. However, no statistical significance was observed in differences of the rs1065489 genotype and allele frequencies among four Gram-negative bacteria and controls. With regard to the three Gram-positive bacteria in this study, there was no statistically significant difference in any CFH polymorphism genotype and allele frequency distribution among cases and controls.

Table 4. Genotype and allele frequency distributions of CFH polymorphisms between different types of infection and healthy controls.

Genotype	Controls n (%)	Gram- negative n (%)	P ₁ value	Gram- positive n (%)	P ₂ value	Mixed G - and G + n (%)	P ₃ value	Fungus n (%)	P ₄ value
rs3753394 C/T									
Total	527	172		31		37		43	
CC	111(21.06%)	32(18.61%)		5(16.13%)		8(21.62%)		6(13.95%)	
CT/TT	416(78.94%)	140(81.39%)	0.488	26(83.87%)	0.651	29(78.38%)	0.936	37(86.05%)	0.267
Allele									
C	474(44.97%)	146(42.44%)		27(43.55%)		33(44.59%)		34(39.53%)	
T	580(55.03%)	198(57.56%)	0.412	35(56.45%)	0.827	41(55.41%)	0.950	52(60.47%)	0.329

rs1065489 G/T									
Total	527	172		31		37		43	
GG	128(24.29%)	43(25.00%)		5(16.13%)		9(24.32%)		8(18.61%)	
GT/TT	399(75.71%)	129(75.00%)	0.851	26(83.87%)	0.388	28(75.68%)	0.996	35(81.39%)	0.401
Allele									
G	507 (48.10%)	165 (47.97%)		30 (48.39%)		33 (44.59%)		34 (39.53%)	
T	547 (51.90%)	179 (52.03%)	0.965	32 (51.61%)	0.965	41 (55.41%)	0.559	52 (60.47%)	0.126
197	P ₁ : healthy control group versus Gram-negative group; P ₂ : healthy control group versus Gram-								
198	positive group; P ₃ : healthy control group versus mixed G - and G + group; P ₄ : healthy control								
199	group versus fungus group.								
200									
201	Table 5. Genotype and allele frequency distributions of CFH polymorphisms between different								
202	types of Gram-negative bacteria and healthy controls.								
		A.		K.		P.			
	Controls	<i>baumannii</i>	P ₁	<i>pneumoniae</i>	P ₂	<i>aeruginosa</i>	P ₃	<i>E. coli</i>	P ₄
Genotype	n (%)	n (%)	value	n (%)	value	n (%)	value	n (%)	value
rs3753394 C/T									
Total	527	111		36		55		54	
CC	111(21.06%)	22(19.82%)		8(22.22%)		5(9.09%)		11(20.37%)	
CT/TT	416(78.94%)	89(80.18%)	0.769	28(77.78%)	0.869	50(90.91%)	0.033	43(79.63%)	0.905
Allele									
C	474(44.97%)	98(44.14%)		29(40.28%)		36(32.73%)		50(46.30%)	
T	580(55.03%)	124(55.86%)	0.822	43(59.72%)	0.438	74(67.27%)	0.014	58(53.70%)	0.792
rs1065489 G/T									
Total	527	111		36		55		54	
GG	128(24.29%)	29(26.13%)		9(25.00%)		9(16.36%)		15(27.78%)	
GT/TT	399(75.71%)	82(73.87%)	0.683	27(75.00%)	0.923	46(83.64%)	0.187	39(72.22%)	0.571
Allele									
G	507(48.10%)	107(48.20%)		30(41.67%)		43(39.10%)		56(51.85%)	
T	547(51.90%)	115(51.80%)	0.979	42(58.33%)	0.290	67(60.90%)	0.072	52(48.15%)	0.458
203	P ₁ : healthy control group versus <i>Acinetobacter baumannii</i> group; P ₂ : healthy control group versus								
204	<i>Klebsiella pneumonia</i> group; P ₃ : healthy control group versus <i>Pseudomonas aeruginosa</i> group;								
205	P ₄ : healthy control group versus <i>Escherichia coli</i> group.								
206	Fisher’s exact test, P ₃ =0.033, OR=2.668, 95%CI (1.072, 6.334) for genotype in rs3753394 .								
207	Chi-squared test, P ₃ =0.014, OR=1.68, 95%CI (1.118, 2.538) for allele in rs3753394 .								
208	BH method, false discovery rate-adjusted P ₃ =0.033 for genotype and allele in rs3753394 ,								
209	respectively.								
210	OR: odds ratio; 95% CI: 95% confidence interval.								
211									

Table 6. Genotype and allele frequency distributions of CFH polymorphisms between different types of Gram-positive bacteria and healthy controls.

Genotype	Controls n (%)	<i>Enterococcus</i> n (%)	P ₁ value	<i>Staphylococcus</i> n (%)	P ₂ value	<i>Streptococcus</i> n (%)	P ₃ value
rs3753394 C/T							
Total	527	12		40		11	
CC	111(21.06%)	3(25.00%)		8(20.00%)		1 (9.09%)	
CT/TT	416(78.94%)	9(75.00%)	0.724	32(80.00%)	0.874	10(90.91%)	0.473
Allele							
C	474(44.97%)	10(41.67%)		38(47.50%)		8(36.36%)	
T	580(55.03%)	14(58.33%)	0.748	42(52.50%)	0.661	14(63.64%)	0.422
rs1065489 G/T							
Total	527	12		40		11	
GG	128(24.29%)	4(33.33%)		9(22.50%)		1(9.09%)	
GT/TT	399(75.71%)	8(66.67%)	0.499	31(77.50%)	0.799	10(90.91%)	0.474
Allele							
G	507(48.10%)	11(45.83%)		40(50.00%)		9(40.91%)	
T	547(51.90%)	13(54.17%)	0.826	40(50.00%)	0.743	13(59.09%)	0.504

P₁: healthy control group versus *Enterococcus* group; P₂: healthy control group versus *Staphylococcus* group; P₃: healthy control group versus *Streptococcus* group.

3.4 Relevance between CFH Polymorphisms and Disease Severity

The average APACHE-II and SOFA scores on Day One, the average days of administration in ICUs, lactate levels and inflammatory values were evaluated for the association of CFH SNPs with disease severity in patients with sepsis. As indicated in Table 7, genotype carriers of CFH polymorphisms rs3753394 C/T, rs1065489 G/T and rs1061170 C/T did not show any statistically significant difference in disease severity of sepsis.

Table 7. Association of CFH Polymorphisms with Disease Severity.

Genotype		APACHE II	SOFA score,	Average hospitalized	Lactate,	Inflammatory Status	
		score, Day 1	Day 1	days in ICU	mmol/L	WBC, 10 ⁹ /L	PCT, ng/ml
rs3753394 C/T	CC	19.59±6.05	8.08±4.16	9.33±8.93	4.33±4.87	17.90±10.97	26.86±46.69
	CT	19.39±6.31	7.67±4.40	11.38±10.82	3.40±3.21	17.59±10.39	25.77±44.84
	TT	19.42±6.76	7.96±3.88	10.77±10.30	3.46±2.99	17.85±9.11	20.06±40.63
P ₁ value		0.967	0.664	0.292	0.176	0.956	0.426
rs1065489 G/T	GG	19.03±5.84	8.11±4.08	10.05±9.57	3.78±3.78	18.29±11.20	29.35±49.18
	GT	19.74±6.57	7.84±4.57	11.37±11.39	3.67±3.78	17.44±10.20	25.25±44.72
	TT	19.28±6.56	7.64±3.57	10.33±8.84	3.42±3.06	17.79±8.96	17.65±36.54

P ₂ value		0.585	0.668	0.484	0.786	0.763	0.132
rs1061170 C/T	CC	21.00±4.24	11.00±4.24	9.00±8.49	1.30±0	15.64±8.00	1.12±1.15
	CT	19.64±6.24	8.22±4.52	9.69±8.86	4.10±5.16	16.36±9.22	28.70±50.67
	TT	19.41±6.43	7.79±4.14	10.89±10.47	3.57±3.32	17.91±10.20	23.62±43.03
P ₃ value		0.915	0.446	0.739	0.560	0.567	0.582

225 APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ
226 Failure Assessment; ICU, Intensive Care Unit; WBC, White Blood Cell Count; PCT,
227 Procalcitonin.

228 P₁ value for genotypes in rs3753394 C/T; P₂ value for genotypes in rs1065489 G/T, P₃ value for
229 genotypes in rs1061170 C/T.

230

231 4. Discussion

232 Identifying genetic variability in genes correlated with pathogenic bacterial infections is
233 essential for elucidating the underlying pathogenesis of sepsis and targeting the closely relevant
234 patients for precise therapeutic interventions. The major objective of this study was to investigate
235 the genetic relevance of functional CFH polymorphisms to sepsis resulting from various
236 pathogenic bacteria. Our main results indicated that the patients carrying CFH rs3753394 T allele
237 had significantly increased risks of suffering from sepsis due to *P. aeruginosa* infection. To the
238 best of our knowledge, this multi-center study was first reported that the functional SNP in CFH
239 gene was significantly associated with the susceptibility to sepsis caused by *P. aeruginosa* in
240 Chinese Han populations.

241 In this research, we confirmed that CFH rs3753394 T allele could significantly elevate the
242 likelihood to develop sepsis because of *P. aeruginosa*, which was consistent with other diseases
243 [14, 26-28]. Our findings in correlation of CFH rs3753394 T allele with *P. aeruginosa*-related
244 sepsis could be supported by the observations that T allele of rs3753394 in CFH gene revealed
245 higher plasma expression levels of CFH [23], but lower serum complement C3 expressions [29].
246 Other results did not identify any significant association between CFH rs1065489 T allele and
247 sepsis induced by *P. aeruginosa*. Yet, T allele of CFH rs1065489 was shown to decrease the risk
248 of meningococcal infection in the European populations [22, 30]. The current study may still
249 support the hypothesis that the dysregulations in the host complement system stimulated by genetic
250 mutations could contribute to the susceptibility to bacterial infections, and thereby facilitate the
251 progression of sepsis [31-33].

252 The underlying mechanism of *P. aeruginosa*-induced sepsis involving CFH SNP is not yet
253 fully understood. Previous studies focused on the interaction of this microbial pathogen with CFH
254 activities may provide possible explanations [34, 35]. *P. aeruginosa* could acquire circulating CFH
255 to the bacterial surface through the elongation factor Tuf to escape host immune responses [34].
256 This organism could also take advantage of host CFH to keep clear of immune attack via

expressing dihydrolipoamide dehydrogenase [35]. Fundamentally, *P. aeruginosa* infection was reported, for the first time, to be significantly related to CFH polymorphism in our study.

P. aeruginosa is an opportunistic Gram-negative pathogen and remains a primary contributor to nosocomial infections in ICUs all over the world by virtue of antibiotics abuse [36, 37]. Antibiotic-resistant *P. aeruginosa* infections may also result in the increased disease severity and the adverse outcomes [38, 39]. In this present study, we did not observe any significant correlation of CFH polymorphisms with disease severity evaluation in patients with sepsis, whereas *P. aeruginosa* still ranked second of all pathogenic bacteria identified in these critically ill patients. Therefore, genotyping and targeting septic patients infected by *P. aeruginosa* at an early stage of the disease may be useful for precisely selecting antibiotics and preventing the progress of antimicrobial resistance.

Limitations to this current study should be considered. Although this multi-center study comprised 1015 consecutive subjects, the sample size was relatively small after divided into different subtypes. Our findings should be verified in a larger cohort of septic patients with *P. aeruginosa* infection. Then, the healthy controls in this study were not matched for age and gender to the septic cases. Thus, age- and gender-matched controls are still required to confirm the results. Last, no additional blood samples could be allowed in this study to analyze the expression levels of CFH and other targeted inflammatory cytokines. Hence, subsequent researches in vivo are still required to investigate the functional effects of CFH polymorphism on CFH activities involved in the process of *P. aeruginosa*-related sepsis.

5. Conclusions

The present study revealed that functional polymorphism rs3753394 in CFH gene was significantly correlated with susceptibility to sepsis due to *P. aeruginosa* infection in Chinese Han populations. CFH SNP rs3753394 may serve as a potential biomarker for targeting *P. aeruginosa* infection in critically ill patients.

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