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SYSTEMATIC REVIEW AND META-ANALYSIS

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No Association of *SERPINE1* −675 Polymorphism With Sepsis Susceptibility

A Meta-Analysis

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Abstract: The serine protease inhibitor clade E member 1 (*SERPINE1*) gene has been suggested to exert great influence on the development of sepsis. But there is little overlap in the results of association between *SERPINE1* −675 4G/5G polymorphism and sepsis.

To get a more precise estimation of this association, we conducted a meta-analysis with a relatively larger sample size including 1806 cases and 2239 controls. Odds ratio (OR) with 95% confidence interval (CI) was used to evaluate the relationship between −675 4G/5G polymorphism and sepsis susceptibility. Subgroup analyses were conducted based on ethnicity and source of controls.

The results showed that there was no association of the *SERPINE1* polymorphism and sepsis susceptibility (5G5G vs 4G4G: OR = 0.87, CI = 0.75–1.03; 5G5G+4G5G vs 4G4G: OR = 0.93, CI = 0.84–1.02; 5G5G vs 4G4G+4G5G: OR = 0.96, CI = 0.83–1.11; 5G vs 4G: OR = 0.94, CI = 0.86–1.01; 4G5G vs 4G4G: OR = 0.90, CI = 0.80–1.01). Nor did any subgroup analysis indicate a significant association.

In conclusion, −675 4G/5G polymorphism in the *SERPINE1* gene may not be associated with the risk of sepsis.

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Abbreviations: CI = confidence interval, CNKI = China national knowledge infrastructure, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, SERPINE1 = serine protease inhibitor clade E member 1, SIRS = systemic inflammatory response syndrome.

INTRODUCTION

Sepsis is a kind of systemic inflammatory response syndrome (SIRS). It results from an immune response triggered by infections induced by bacteria, fungi, parasites, or viruses, with the primary infection more frequently seen in abdominal organs, brain, lungs, urinary tract, and skin.[1–3] Common symptoms and signs of this whole-body inflammation include fever, polypnea, flushed skin, high heart rate, increased breathing rate, low blood pressure, or confusion.[1] Despite the advance in effective antibiotics and supportive care, sepsis has a high mortality in critically ill patients.[4] Identifying the predictive markers will help to better elucidate the disease pathogenesis. A series of genetic studies have investigated the role of serine protease inhibitor clade E member 1 (*SERPINE1*) in the pathogenesis of sepsis.[5] *SERPINE1,* also named plasminogen activator inhibitor-1 and *PAI-1*, is located on chromosome 7 (7q21.3-q22) in humans. It is a key member of serine proteinase inhibitor super family and serves as a main regulator of tissue and urinary plasminogen activators and fibrinolysis inhibitors.[6,7] Results of previous research have established a linkage between the *SERPINE1* gene and risk of sepsis.[8] This finding was confirmed in recent reports, which also demonstrated high concentrations of *SERPINE1* in patients sustaining meningococcal sepsis.[9,10]

There lies a single nucleotide insertion/deletion polymorphism (−675 4G/5G) in the promoter region. The recently described common promoter polymorphism is important in the transcription of *SERPINE1* and the 4G allele has been correlated with higher plasma *SERPINE1* activity.[11] In addition, the 4G allele was reported to increase the risk of sepsis and related inflammation diseases such as pneumonia and trauma.[12–18]

Among the published studies, there is little overlap in the results concerning the association between *SERPINE1* −675 4G/5G and sepsis. Herein, meta-analysis was used to provide compelling evidence.

METHODS

Literature Search

We conducted a systematic computerized literature search of the PubMed, EMBASE, Science Direct, China national knowledge infrastructure (CNKI) and WanFang databases up to November 2014, to identify all articles that investigated the association of *SERPINE1* −675 4G/5G polymorphism and sepsis, without language restrictions. Combinations of the following key words *SERPINE1* or *PAI-1*, ''polymorphism'' or ''variant'' and ''sepsis'' were used. We also handsearched the references lists of all related review articles and the original articles considered eligible for the meta-analysis. In the meanwhile, the study was supported by the Research Ethics Committee of General Hospital of Beijing Military Region.

Inclusion and Exclusion Criteria

The studies were selected according to the following criteria: (a) evaluated the relationship between −675 4G/5G polymorphism and the risk of sepsis; (b) a case-control or cohort study; (c) provided sufficient data to calculate an odds ratio (OR) with 95% confidence interval (CI). Genetic association studies focusing on sepsis patients only or reporting limited data on genotype frequency were considered ineligible. Whenever

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several articles were overlapped in terms of subjects, only the largest article with complete genetic data was included in this meta-analysis.

Data Extraction

Data on name of first author, publication year, country, ethnicity, control source, genotyping method, numbers of cases and controls, genotype and allele frequencies in cases and controls and *P* value of Hardy–Weinberg equilibrium (HWE) were extracted by 2 investigators and subsequently checked by a 3rd investigator to assure data accuracy. The divergences, if any, were settled by discussion including a senior investigator.

Statistical Analysis

Fisher's test was adopted to test HWE in controls and $P < 0.05$ was considered significant disequilibrium. ORs with 95% CI were used to assess the relationship of *SERPINE1* −675 4G/5G polymorphism with sepsis. Pooled ORs were calculated for 5G5G versus 4G4G, 5G5G+4G5G versus 4G4G, 5G5G versus 4G4G+4G5G, 5G versus 4G and 4G5G versus 4G4G, assuming homozygous, dominant, recessive, additive, and heterozygous genetic models, respectively. Both overall analyses and subgroup analyses based on ethnicity and source of controls were performed. The heterogeneity across the studies included in the meta-analysis was examined by the χ^2-based *Q*-test, with the significance level defined at $P < 0.05$. In a case of significant heterogeneity, pooled ORs were calculated by the random-effects model; otherwise, the fixed-effects model was used. Potential publication bias was checked by Begg's funnel plot and further evaluated by Egger's weighted regression test. Sensitivity analysis was used to assess the stability of results. All statistical analyses were performed using Stata software (Version 12.0).

RESULTS

Characteristics of the Studies

As presented in Figure 1, 112 citations were identified through PubMed, EMBASE, and Science Direct databases and 27 additional citations were obtained from CNKI and WanFang. By scanning the title and abstract, we excluded 63 records with various reasons. We retrieved 76 full-text articles and examined their eligibility. Among these, 64 articles were excluded, because 14 focused on sepsis treatment, 3 used the same case series, 26 were designed as case-only studies, and 21 provided insufficient genotype data. Ultimately, 12 studies[10,11,15,17,19–26] with 1806 cases and 2239 controls were combined in the meta-analysis. As Table 1 shows, 2 studies were conducted in Asian population and 10 in Caucasian samples. In addition, we included 4 hospital-based studies and 8 population-based studies.

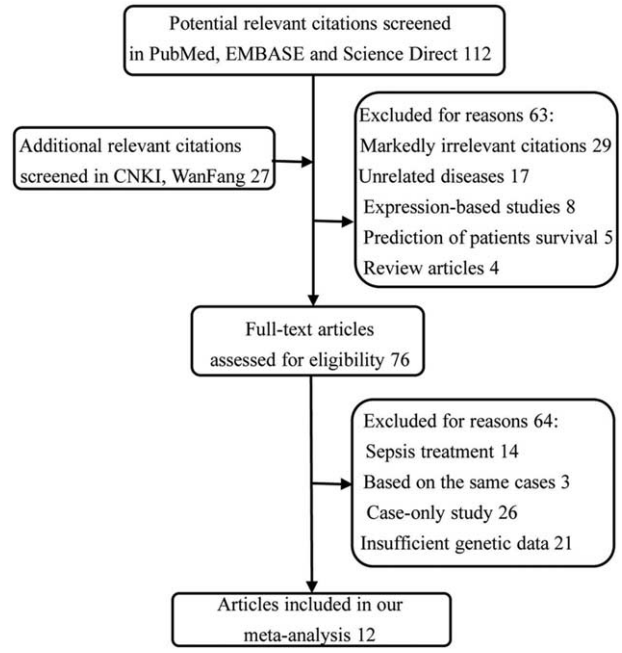

FIGURE 1. Flow diagram of study selection in the meta-analysis.

Meta-Analysis

Using the fixed-effects homozygous model, we found individuals with the 5G5G genotype in comparison to individuals with 4G4G genotype had a decreased risk of sepsis, though

TABLE 1. Characteristics of Eligible Studies in the Meta-analysis

First Author	Year	Country	Ethnicity	Control Source	Genotyping Method	Case	Control	HWE
Wei	2008	China	Asian	Population-based	Allele-specific PCR	148	181	0.91
Wingeyer	2012	Argentina	Caucasian	Population-based	PCR	166	214	0.31
Segarra	2007	Spain	Caucasian	Population-based	Sequencing	165	80	0.62
Wingeyer	2009	Argentina	Caucasian	Population-based	PCR	166	136	0.51
Westendop	1999	Netherlands	Caucasian	Population-based	PCR	85	131	0.51
Sipahi	2006	Turkey	Caucasian	Population-based	PCR	42	113	0.93
Menges	2001	UK	Caucasian	Population-based	PCR	29	32	0.71
Geishofer	2005	Central Europe	Caucasian	Population-based	Allele-specific PCR	137	316	0.31
Hermans	1999	UK, Netherlands	Caucasian	Hospital-based	PCR	154	226	0.89
Henckaerts	2009	Denmark	Caucasian	Hospital-based	PCR	395	555	0.69
Haralambous	2003	UK	Caucasian	Hospital-based	Allele-specific oligo melting/SNaPshot	230	155	0.65
Zhan	2005	China	Asian	Hospital-based	PCR	89	100	1

HWE = Hardy–Weinberg Equilibrium, PCR = Polymerase Chain Reaction.

TABLE 2. *SERPINE1* −675 4G/5G Polymorphism and Sepsis Risk

		5G5G Versus 4G4G		5G5G+4G5G Versus 4G4G		5G5G Versus 4G4G+4G5G		5G Versus 4G		4G5G Versus 4G4G	
		OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h
Ethnicity	Asian	0.75 (0.48, 1.17)	0.202	0.87 (0.66, 1.14)	0.428	0.84 (0.55, 1.27)	0.228	0.86 (0.69, 1.07)	0.200	0.84 (0.61, 1.16)	0.504
	Caucasian	0.89 (0.75, 1.06)	0.498	0.94 (0.84, 1.04)	0.955	0.98 (0.84, 1.15)	0.167	0.95 (0.87, 1.03)	0.414	0.91 (0.80, 1.03)	0.961
Control Source	Population	0.91 (0.74, 1.13)	0.308	0.93 (0.81, 1.07)	0.890	1.03 (0.85, 1.26)	0.108	0.96 (0.86, 1.07)	0.245	0.89 (0.76, 1.05)	0.931
	Hospital	0.83 (0.65, 1.06)	0.607	0.92 (0.80, 1.06)	0.771	0.87 (0.70, 1.09)	0.634	0.91 (0.81, 1.02)	0.579	0.91 (0.77, 1.07)	0.739
Total		0.87 (0.75, 1.03)	0.485	0.93 (0.84, 1.02)	0.967	0.96 (0.83, 1.11)	0.191	0.94 (0.86, 1.01)	0.400	0.90 (0.80, 1.01)	0.977

CI = confidence interval, OR = odds ratio, P_h = *P*-value of heterogeneity test.

the value did not reach the significance threshold (OR = 0.87, CI = 0.75–1.03) (Table 2). Likewise, other models tested did not indicate a significant association between *SERPINE1* −675 4G/5G polymorphism and the risk for sepsis (5G5G+4G5G vs. 4G4G: OR = 0.93, CI = 0.84–1.02; 5G5G vs. 4G4G+4G5G: OR = 0.96, CI = 0.83–1.11; 5G vs. 4G: OR = 0.94, CI = 0.86–1.01; 4G5G vs. 4G4G: OR = 0.90, CI = 0.80–1.01) (Table 2).

We proceeded to subgroup analyses and no major effects were seen in any subgroup. Figures 2 and 3 illustrate the forest plots constructed for the homozygous model after stratification by ethnicity and source of controls, respectively.

Sensitivity Analysis and Publication Bias Test

Sensitivity analysis was carried out by deleting the single studies one by one to check their influence on the combined values. No substantial changes were seen in the pooled values. This suggested the stability of our meta-analysis results (data not shown). Symmetrical distribution was indicated in the funnel plots. More importantly, no evidence of high possibility of publication bias was found using Egger's weighted regression test ($P = 0.105$). Figure 4 shows the funnel plot constructed for the homozygous model.

DISCUSSION

SERPINE1 is considered to be a vital factor in the deregulation of fibrinolysis during the activity of coagulation in sepsis. It is suggested that increased plasma levels of *SERPINE1* result in multiple organ dysfunction and subsequently increase the mortality of sepsis.[27–31] Plasma *SERPINE1* concentrations are regulated by various factors. A functional polymorphism, −675 4G/5G, situated in the promoter region of the *SERPINE1* gene has been described as an important regulator of plasma *SERPINE1* levels. The presence of −675 4G/5G genotypes has been suggested to raise serum *SERPINE1* concentrations, with the 4G/4G genotype associated with the highest levels, followed by the 5G/5G genotype, and the heterozygous genotype.[32] It is this significant effect on serum concentrations of *SERPINE1* that leads to much speculation of a causal association between the −675 4G/5G polymorphism and sepsis.

Recently, a number of studies representing various populations have been conducted to investigate the relationship between *SERPINE1* −675 4G/5G polymorphism and the genetic risk of sepsis.[10,15,17,19,20] The results, however, are controversial rather than conclusive. For example, a case-control study investigated multiple candidate genes among Turkish samples (42 sepsis cases and 113 controls), suggesting that the −675 4G/5G polymorphism is a susceptibility site for severe

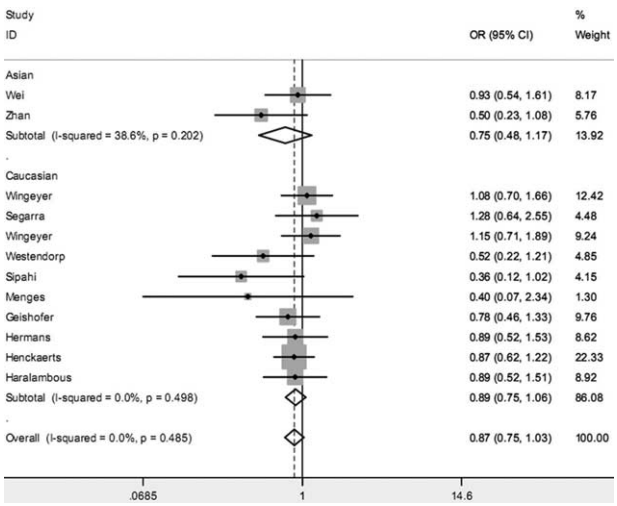

FIGURE 2. Forest plot of association between *SERPINE1* −675 4G/5G polymorphism and sepsis risk under the homozygous model by ethnicity.

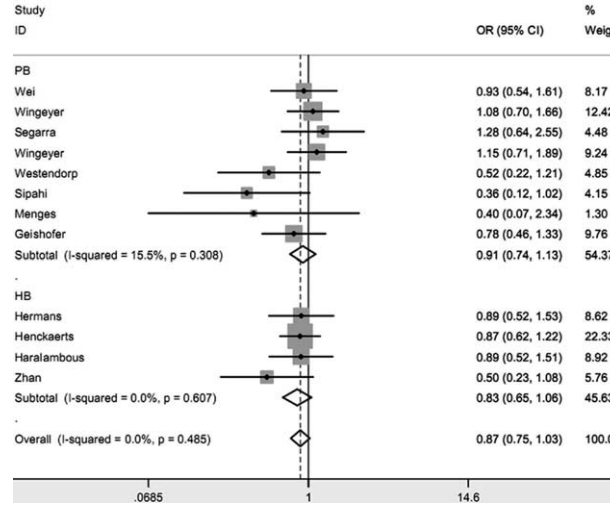

FIGURE 3. Forest plot of association between *SERPINE1* −675 4G/5G polymorphism and sepsis risk under the homozygous model by the control source.

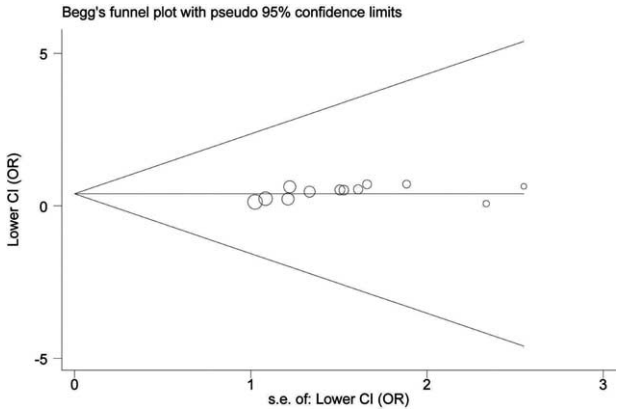

FIGURE 4. Funnel plot constructed for the homozygous model.

sepsis in children.[21] The finding is consistent with a Caucasian study based on adult sepsis patients and an Asian study among children.[12,25] In contrast, in a replication study by Zhan et al, the results suggested a significant association both at the genotypic and the allelic level among 89 cases and 100 controls of Han Chinese descent.[22] More recently, Geishofer et al and Wingeyer et al lend further support for an increased risk of sepsis in relation to the presence of the 4G/4G genotype.[20,23] The inconsistency either caused by heterogeneous populations or sampling variance requires a meta-analysis to compromise.

In our article, we demonstrated evidence that the presence of the −675 4G/5G polymorphism was not associated with genetic risk of sepsis. The present finding seems to contradict previous studies where the promoter polymorphism was shown to increase plasma *SERPINE1* levels.[32] However, it is likely that the −675 4G/5G polymorphism is a low-penetrance site and the minor effect can be detected in a larger analysis. It is also possible that the genetic predisposition to sepsis differs among individuals of different ancestries. Additional research with a larger sample and a variety of ethnic groups is clearly needed to investigate the aforementioned possibilities.

Several points should be concerned in interpreting the obtained results. First, previous research suggested significantly higher plasma *SERPINE1* concentrations in patients with sepsis ascribed to the presence of the 4G allele or the 4G4G genotype.[10] This implicates that the enhanced transcriptional activity induced by the mutation in the *SERPINE1* promoter may represent a mechanism underlying the elevated levels of *SERPINE1* in sepsis patients. But our meta-analysis did not show any evidence supporting an association. The most likely reason might relate to the limited sample size. Second, unadjusted ORs were used to estimate the genetic risk of sepsis. Common confounding factors such as the age and the gender were not considered. Third, we included some small studies. Therefore, publication bias may have been introduced, although the tests we utilized did not suggest such bias.

In conclusion, our meta-analysis results suggest that −675 4G/5G polymorphism in the promoter region of *SERPINE1* gene may not contribute to the development of sepsis. Additional analyses are warranted in this chromosomal region to determine the association between the polymorphism site being investigated and incidence of sepsis in various ethnic populations.

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