Association between IL-6 polymorphisms and sepsis

Peiyang Hu[1] (iD), Yimin Chen[2], Jianliang Pang[2] and Xionghuan Chen[3]

Abstract

The aim of the present study was to determine whether IL-6 polymorphisms correlate with sepsis. According to the inclusion criteria, the association of IL-6 polymorphisms with sepsis was searched in databases and analysed using comprehensive meta-analysis software. A total of 16 studies were included in this meta-analysis. There was no significant association between the IL-6-174G/C polymorphism and sepsis risk in the total population (C vs. G: OR = 1.04, 95% CI = 0.79–1.38; CC vs. GG: OR = 0.86, 95% CI = 0.53–1.41; CG vs. GG: OR = 0.99, 95% CI = 0.79–1.24; dominant model: OR = 0.97, 95% CI = 0.74–1.29; recessive model: OR = 0.92, 95% CI = 0.61–1.39). When patients were stratified according to ethnicity, a statistically significant association was observed in Asians and Africans. As for the -572G/C polymorphism, the results showed that the IL-6-572C/G polymorphism was not associated with sepsis susceptibility (G vs. C: OR = 0.98, 95% CI = 0.79–1.22; GG vs. CC: OR = 1.46, 95% CI = 0.53–4.03; GC vs. CC: OR = 0.82, 95% CI = 0.54–1.27; dominant model: OR = 0.88, 95% CI = 0.55–1.41; recessive model: OR = 1.55, 95% CI = 0.82–2.92). The data indicated that the IL-6-174G/C polymorphism may contribute to sepsis risk, especially in Africans and Asians. No significant association was observed between the IL-6-572G/C polymorphism and sepsis risk.

Introduction

Sepsis is considered to be a complicated clinical syndrome arising from systemic inflammatory reactions to bacteria or bacterial products.[1] Although there has been great improvement in antibiotic therapy and life support, the global death rate of sepsis patients remains up to 30–60%.[2] Therefore, predictive markers to identify high-risk populations are urgently needed for early detection and preventive care. Cytokines are critically involved in modulation of host immune response, while changed cytokine levels have been shown to participate in sepsis development.[3] Delineating the variation in gene expression and associated differences in responses to sepsis might facilitate in exploiting new genetically tailored diagnostic and therapeutic interventions, which could improve outcome in patients with sepsis susceptibility.

IL-6 is a vital inflammatory cytokine generated by various cells, such as leucocytes, endothelial cells, myocytes, adipocytes and fibroblasts.[4] IL-6 plays a significant role in the immune response and regulation of inflammation reactions. High IL-6 levels have been demonstrated to be related to the elevated risk and death rate of severe sepsis.[5] The IL-6 gene, located on 7p21 and spanning 5 kb, contains four introns and five exons. There have been several identified polymorphisms in the IL-6 gene promoter region. Among these, the common -174G/C polymorphism (rs1800795) contains a DNA-binding site for nuclear factor IL-6, a transcription factor which can interact with estradiol receptor complexes to regulate IL-6 gene expression.[6]

[1]Department of Traumatology, Tiantai People's Hospital, PR China
[2]Intensive Care Unit, Tiantai People's Hospital, PR China
[3]Trauma Centre, Tiantai People's Hospital, PR China

Corresponding author:
Peiyang Hu, Department of Traumatology, Tiantai People's Hospital, Tiantai, Zhejiang 317200, PR China.
Email: hu_peiyang@126.com

The -572G/C polymorphism (rs1800796) does not contain any potent homology to any identified transcription factor-binding site, with a close location to glucocorticoid response elements.[7] To date, there have been much research evaluating the association of IL-6 polymorphisms with sepsis, albeit with inconsistent findings.

Meta-analysis is a useful method to summarise the different articles. It not only overcomes the drawbacks of small sample size and inadequate statistical power of genetic studies with complicated characteristics, but also provides more convincing conclusions compared to a single study.[8] Thus, in this research study, a meta-analysis was conducted by enrolling eligible studies to assess the relationship between IL-6-174G/C and -572G/C polymorphisms and sepsis.

Materials and methods

Literature search

We thoroughly searched the online electronic databases (PubMed, EMBASE and CNKI) using the following search terms: (interleukin-6 or IL-6) and (polymorphism or variant or variation) and ('sepsis'). In addition, we manually searched the references of original studies and reviews on this topic. There was no restriction on language.

Criteria for inclusion and exclusion

Eligible studies were enrolled if they met the following criteria: (a) case-control studies, (b) only cases with definitive diagnosis of sepsis and (c) evaluation of IL-6 polymorphisms and sepsis. Exclusion criteria were: (a) abstracts, reviews and duplication of studies; (b) not case-control studies; and (c) absence of genotype frequency.

Data extraction

The following information was extracted from every article by two independent authors: year of publication, first author, ethnicity, numbers as well as genotype numbers of cases and controls, with evidence of Hardy–Weinberg equilibrium (HWE) in controls. Discrepancies were resolved by discussion.

Statistical analysis

Stata v12.0 (Stata Corp., College Station, TX) was used for statistical analysis. ORs with corresponding 95% CIs was used to assess the correlation of IL-6 gene polymorphisms (-174G/C and -572G/C) with sepsis under A versus a, homozygote (AA vs. aa), heterozygote comparison (AA vs. Aa) and dominant model and recessive model between groups. To be specific, the dominant model referred to as $Aa + aa$ versus AA, where 'A' and 'a' represented major and minor alleles, respectively, and the recessive model was aa versus $AA + Aa$.[9] Moreover, the variation caused by heterogeneity was estimated by calculating the inconsistency index I^2, with I^2 values of 25%, 25–75% and 75% representing low, moderate or high degrees of inconsistency, respectively.[10] When the heterogeneity was absent ($I^2 < 50\%$), a fixed-effects model was used to calculate the combined OR estimates for every study; otherwise, a random-effects model was applied. Additionally, sensitivity analysis was conducted by limiting the meta-analysis to research conforming to HWE. Finally, possible publication bias was visually determined by Begg's funnel plots.[11]

Results

Characteristics of included studies

The study selection process is shown in Figure 1. According to the inclusion criteria, 16 qualified case-control studies were finally enrolled,[12–27] all of which assessed the correlation of IL-6 polymorphisms with sepsis risk. All studies were published in English. Fifteen articles studied the -174G/C polymorphism and four studied the -572C/G polymorphism. For -174G/C, there were 11 studies with Caucasian subjects, two with Asian populations, two with African populations and one with a mixed population. Eight studies were performed in adults, six in neonates and two in paediatric populations. For -572C/G, there were two studies with Caucasian subjects and two with Asian populations. Three studies were conducted in adults and one in paediatric populations. The major features of enrolled studies are summarised in Table 1.

Meta-analysis results

The major outcomes of the meta-analysis of the IL-6-174G/C polymorphism and sepsis risk are presented in Figure 2 and Table 2. Overall, the -174G/C polymorphism was not significantly associated with sepsis risk (C vs. G: OR = 1.04, 95% CI = 0.79–1.38; CC vs. GG: OR = 0.86, 95% CI = 0.53–1.41; CG vs. GG: OR = 0.99, 95% CI = 0.79–1.24; dominant model: OR = 0.97, 95% CI = 0.74–1.29; recessive model: OR = 0.92, 95% CI = 0.61–1.39). In subgroup analysis stratified by ethnicity, a significant association was detected in Asians and Africans but not in Caucasians. In subgroup analysis by age,-174G/C was insignificantly related to sepsis risk in adults, neonates and paediatric populations. On subgroup analysis stratified by HWE, eight papers in Caucasians were included with HWE studies, and the analyses on their
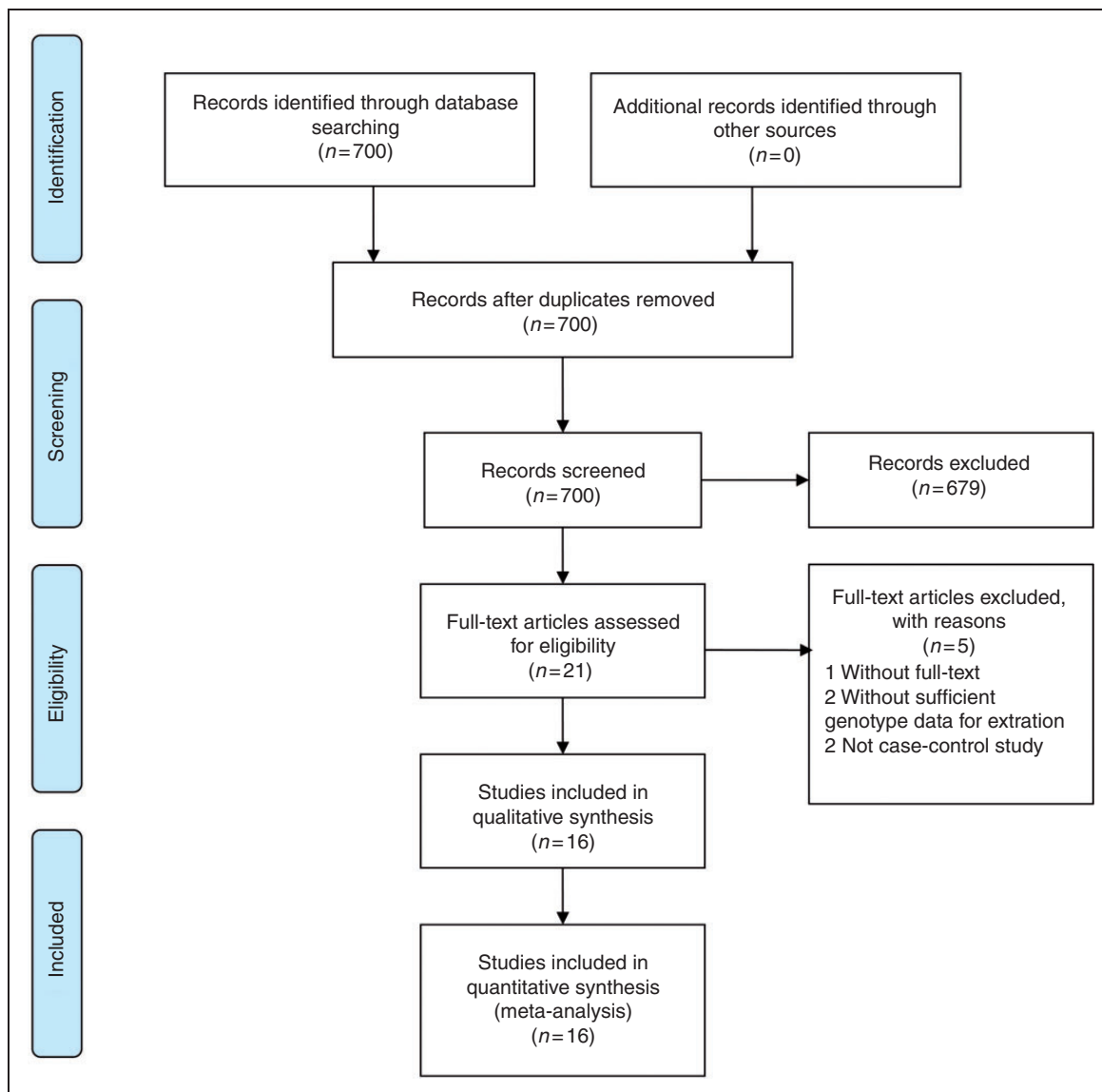
Figure 1. Flow diagram for selection of publication.

cumulative data indicate that the IL-6-174G/C polymorphism is not associated with sepsis risk (C vs. G: OR = 1.35, 95% CI = 0.89–2.03; CC vs. GG: OR = 0.53, 95% CI = 0.25–1.12; CG vs. GG: OR = 0.93, 95% CI = 0.65–1.31; dominant model: OR = 0.77, 95% CI = 0.50–1.18; recessive model: OR = 0.62, 95% CI = 0.35–1.09). In addition, sensitivity analysis was conducted through omitting HWE studies. Non-HWE studies changed the final results, implicating the existence of publication bias in results due to non-HWE studies. The major findings of the IL-6-572C/G polymorphism and sepsis risk are listed in Figure 3 and Table 3. Combined results revealed that the IL-6-572C/G polymorphism was not related to sepsis susceptibility (G vs. C: OR = 0.98, 95%

CI = 0.79–1.22; GG vs. CC: OR = 1.46, 95% CI = 0.53–4.03; GC vs. CC: OR = 0.82, 95% CI = 0.54–1.27; dominant model: OR = 0.88, 95% CI = 0.55–1.41; recessive model: OR = 1.55, 95% CI = 0.82–2.92). On subgroup analysis stratified by ethnicity, the IL-6-572C/G polymorphism was not statistically associated with sepsis risk in Asians or other ethnic groups. When patients were stratified according to age, this polymorphism was not related to sepsis risk in adults.

Publication bias

Begg's funnel plots were conducted to evaluate the publication bias of enrolled articles. There was no

Table 1. Study selection and subject characteristics of included studies in meta-analysis.

| Author	Yr	Ethnicity	Age	Cases	Controls	Genotypes for cases G	Genotypes for cases C	Genotypes for controls G	Genotypes for controls C	Genotypes for cases GG	Genotypes for cases GC	Genotypes for cases CC	Genotypes for controls GG	Genotypes for controls GC	Genotypes for controls CC	P for HWE
-174G/C																
Schluter	2002	Caucasian	Adult	50	276	51	49	313	239	13	25	12	91	131	54	0.58
Treszl	2003	Caucasian	Neonatal	33	70	49	17	97	43	18	13	2	34	29	7	0.82
Balding	2003	Caucasian	Adult	183	389	215	151	371	241	59	97	27	97	177	32	0.00
Ahrens	2004	Caucasian	Neonatal	50	306	69	31	57	309	24	21	5	3	51	129	0.42
Sipahi	2006	Caucasian	Pediatric	44	77	66	22	123	31	26	14	4	52	19	6	0.04
Gopel	2006	Caucasian	Neonatal	97	320	108	86	399	241	29	50	18	128	143	49	0.39
Baier #1	2006	African	Neonatal	114	119	206	22	229	9	94	18	2	110	9	0	0.67
Baier #2	2006	Caucasian	Neonatal	31	26	40	22	30	22	12	16	3	7	16	3	0.01
Michalek	2007	Caucasian	Pediatric	409	644	477	341	665	623	127	223	59	160	345	139	0.07
Abdel-Hady	2009	African	Neonatal	54	71	60	48	88	54	17	26	11	28	32	11	0.71
Davis	2010	Caucasian	Adult	23	52	31	15	64	40	10	11	2	21	22	9	0.44
Carregaro	2010	Mix	Adult	97	207	137	57	284	130	49	39	9	94	96	17	0.27
Shimada	2011	Asian	Adult	123	102	246	0	204	0	123	0	0	102	0	0	0.00
Martin-Loeches	2012	Caucasian	Adult	1227	953	1678	776	1289	617	581	516	130	438	413	102	0.75
Panayides	2015	Caucasian	Adult	185	108	303	67	184	32	123	57	5	79	26	3	0.63
Mao	2016	Asian	Adult	188	162	149	227	182	142	56	37	95	68	46	48	0.00
-572C/G																
Michalek	2007	Caucasian	Pediatric	409	644	51	767	56	1228	7	37	365	2	52	588	0.46
Gu	2008	Asian	Adult	54	321	26	82	153	489	5	16	33	16	121	184	0.49
Shimada	2011	Asian	Adult	123	102	53	191	41	163	3	47	72	2	37	63	0.19
Panayides	2015	Caucasian	Adult	198	115	49	347	49	181	6	37	155	6	37	72	0.66
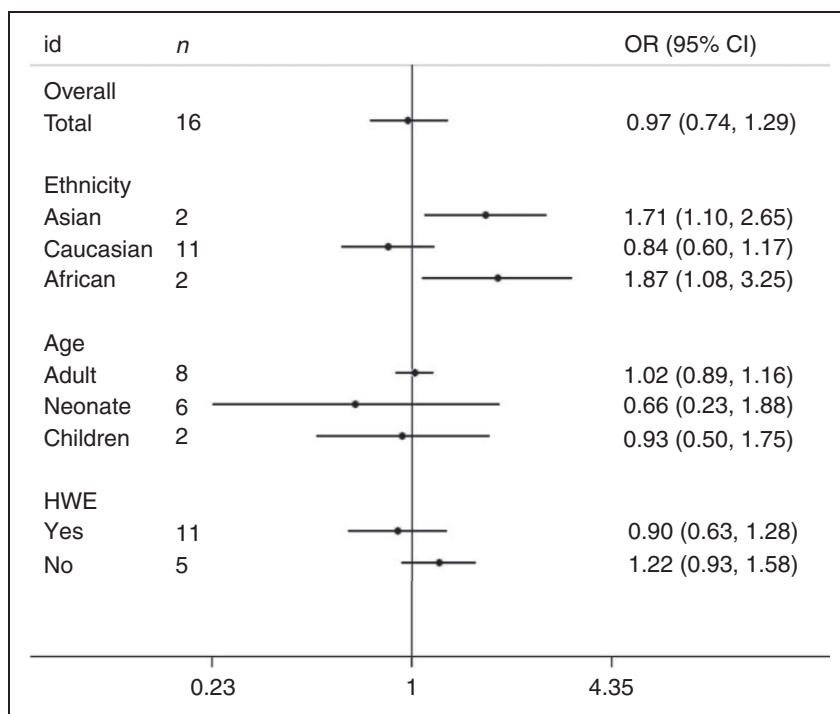
Figure 2. Forest plot of OR for association between the IL-6-174G/C polymorphism and sepsis under the dominant model.

Table 2. Summary ORs and 95% CIs of IL-6-174G/C polymorphism and sepsis risk.

	n	C vs. G OR (95% CI)	CC vs. GG OR (95% CI)	GC vs. GG OR (95% CI)	Dominant model OR (95% CI)	Recessive model OR (95% CI)
Total	16	1.04 (0.79–1.38)	0.86 (0.53–1.41)	0.99 (0.79–1.24)	0.97 (0.74–1.29)	0.92 (0.61–1.39)
Ethnicity						
Asian	2	0.51 (0.38–0.69)	2.40 (1.46–3.94)	0.94 (0.72–1.24)	1.71 (1.10–2.65)	2.43 (1.56–3.77)
Caucasian	11	1.22 (0.91–1.63)	0.66 (0.37–1.19)	0.97 (0.52–1.80)	0.84 (0.60–1.17)	0.74 (0.46–1.18)
African	2	0.61 (0.40–0.93)	1.96 (0.76–5.10)	1.75 (0.98–3.11)	1.87 (1.08–3.25)	1.63 (0.69–3.87)
Age						
Adult	8	0.87 (0.69–1.09)	1.21 (0.98–1.50)	0.97 (0.84–1.11)	1.02 (0.89–1.16)	1.31 (0.91–1.88)
Neonate	6	1.29 (0.48–3.46)	0.50 (0.08–3.04)	0.79 (0.35–1.80)	0.66 (0.23–1.88)	0.66 (0.18–2.51)
Children	2	1.08 (0.65–1.81)	0.57 (0.40–0.83)	0.87 (0.66–1.14)	0.93 (0.50–1.75)	0.64 (0.46–0.88)
HWE						
Yes	11	1.15 (0.82–1.61)	0.70 (0.38–1.28)	1.00 (0.75–1.34)	0.90 (0.63–1.28)	0.75 (0.46–1.21)
No	5	0.78 (0.52–1.17)	1.79 (1.25–2.57)	0.95 (0.71–1.28)	1.22 (0.93–1.58)	1.88 (1.36–2.60)
Caucasian (yes)	8	1.35 (0.89–2.03)	0.53 (0.25–1.12)	0.93 (0.65–1.31)	0.77 (0.50–1.18)	0.62 (0.35–1.09)
Caucasian (no)	3	0.93 (0.74–1.17)	1.28 (0.76–2.18)	0.94 (0.67–1.34)	1.00 (0.72–1.40)	1.38 (0.85–2.24)

HWE: Hardy–Weinberg equilibrium.

obvious asymmetry in the allele model in consideration of the shapes of funnel plots in all genetic models, implying the low publication bias in our meta-analysis (Figures 4 and 5).

Discussion

Sepsis is characterised as a systemic inflammatory response due to infection. A central event in the pathophysiological cascade of sepsis is excessive systemic release of pro-inflammatory cytokines such as IL-6 in response to microbial invasion. IL-6 is a multifunctional cytokine regulating immune reactions and is regarded as an endogenous pyrogen that induces fever in patients with infections. The -174G/C and -572C/G polymorphisms in the IL-6 gene have been found to be related to IL-6 promoter activity, resulting in interindividual alterations in cytokine levels.[28] In various
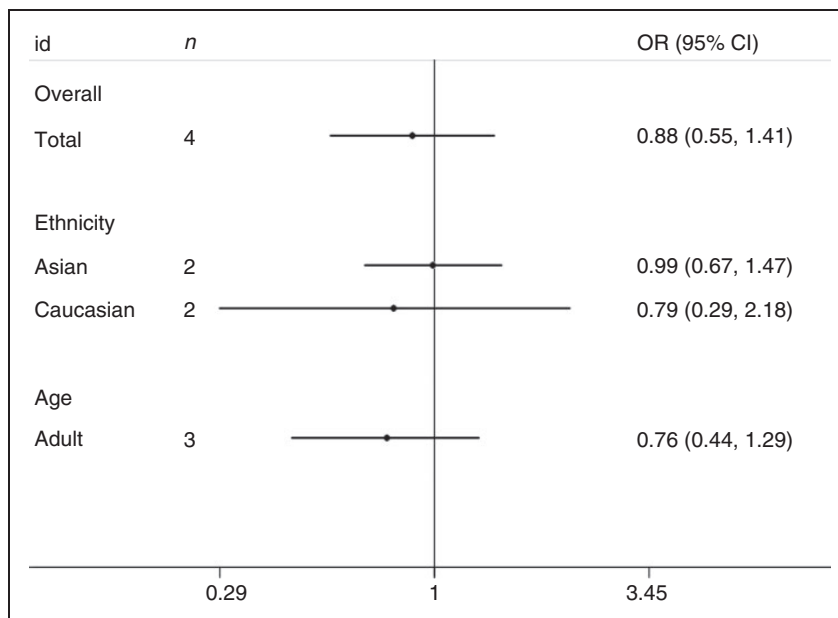
Figure 3. Forest plot of OR for association between the IL-6-572G/C polymorphism and sepsis under the dominant model.

Table 3. Summary ORs and 95% CIs of IL-6-572C/G polymorphism and sepsis risk.

	n	G vs. C OR (95% CI)	GG vs. CC OR (95% CI)	GC vs. CC OR (95% CI)	Dominant model OR (95% CI)	Recessive model OR (95% CI)
Total	4	0.98 (0.79–1.22)	1.46 (0.53–4.03)	0.82 (0.54–1.27)	0.88 (0.55–1.41)	1.55 (0.82–2.92)
Ethnicity						
Asian	2	1.06 (0.76–1.47)	1.61 (0.63–4.08)	0.93 (0.62–1.41)	0.99 (0.67–1.47)	1.71 (0.68–4.32)
Caucasian	2	0.88 (0.32–2.40)	1.53 (0.13–17.95)	0.74 (0.31–1.79)	0.79 (0.29–2.18)	1.67 (0.18–15.81)
Age						
Adult	3	0.83 (0.52–1.34)	0.98 (0.47–2.06)	0.72 (0.43–1.22)	0.76 (0.44–1.29)	1.10 (0.53–2.28)
Paediatric	1	/	/	/	/	/

genetic studies, IL-6 polymorphisms in the promoter region have been shown to increase the risk for sepsis, albeit with inconsistent results. Different ethnicities, insufficient power and the small effect of the polymorphism on sepsis risk make it even more complicated to interpret these studies. In consideration of the elevated numbers of studies in recent years, there is a need to reconcile these data.

In this meta-analysis, we systemically examined the correlation of IL-6 gene polymorphisms (-174G/C and -572C/G) with sepsis susceptibility. As a result, the IL-6 gene -174G/C polymorphism was not statistically associated with sepsis risk. In subgroup analysis stratified by nationality, this polymorphism was related to sepsis in Asians and Africans but not in Caucasians. On the one hand, only two articles on Asians and Africans were analysed, and the number of studies and samples were relatively small. On the other hand,

two included studies for Asian ethnicity were not in HWE equilibrium. However, the included studies report very limited information to provide any detailed insight into these potential problems. Well-designed studies with more subjects will be required for further validation of these results. In subgroup analysis stratified by age, this polymorphism was not significantly correlated to sepsis risk in adult, neonatal or paediatric populations. Sensitivity analysis was conducted by omitting HWE studies for the total population and Caucasian ethnicity. Non-HWE studies changed the outcomes, implicating that our results had a publication bias due to non-HWE studies. For the IL-6-572C/G polymorphism, there was no significant relationship of this genotype with sepsis risk, which was still insignificant in subsequent subgroup analyses stratified by ethnicity or age. As with other diseases, the pathogenesis of sepsis is caused by synergistic
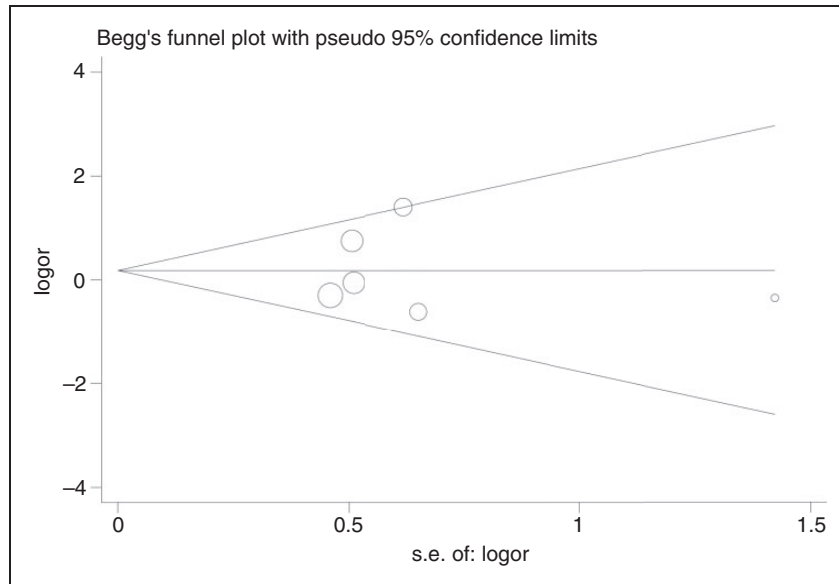
Figure 4. Begg's funnel plot for association between the IL-6-174G/C polymorphism and sepsis.
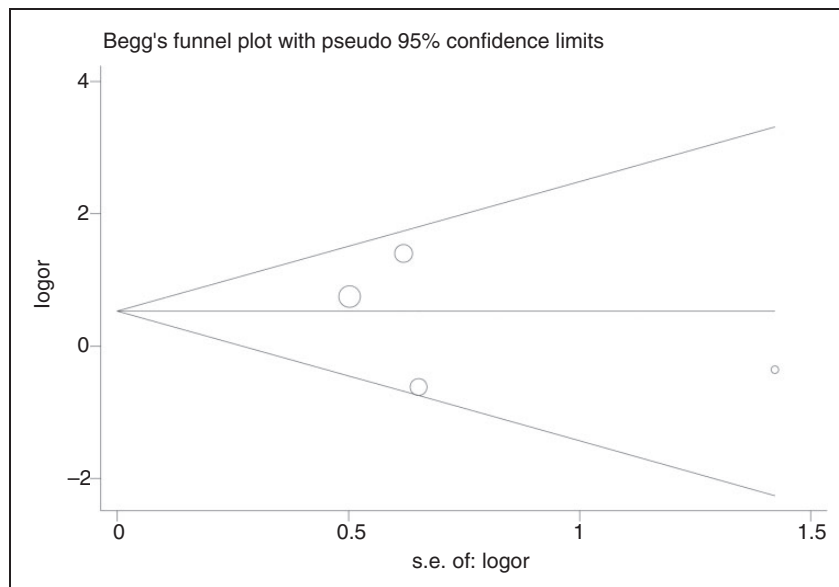

Figure 5. Begg's funnel plot for association between the IL-6-572G/C polymorphism and sepsis.

interaction of several genes and gene environments, and these interactions require further investigation in future studies.

There are certain limitations in the study. First, the majority of included research only studied the correlation of IL-6 polymorphisms with sepsis risk, while there is inaccessibility of more accurate adjusted ORs for other covariates (including family history, age, environmental factors and infectious condition). Second, the incomplete raw data and publication limitations led to a failure to enrol all relevant studies in the present meta-analysis. Third, we failed to investigate the genetic–environmental interplays due to multiple testing and inadequate studies. Indeed, it is probable that certain lifestyle and environmental factors (such as smoking) could affect the relationship of gene polymorphisms with sepsis risk.

Taken together, we demonstrated that the IL-6-174G/C polymorphism may contribute to sepsis risk in Africans and Asians. No significant association was observed between the IL-6-572G/C polymorphism

and sepsis risk. Further evaluation of the influence of gene polymorphisms on sepsis will require well-designed studies with large sample sizes.

Declaration of conflicting interests

Funding

ORCID iD

Peiyang Hu https://orcid.org/0000-0002-5468-7798

References

1. Rittirsch D, Flierl MA and Ward PA. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 2008; 8: 776–787.
2. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. *Physiol Rev* 2013; 93: 1247–1288.
3. Grealy R, White M, Stordeur P, et al. Characterising cytokine gene expression signatures in patients with severe sepsis. *Mediators Inflamm* 2013; 2013: 164246.
4. Van Snick J. Interleukin-6: an overview. *Annu Rev Immunol* 1990; 8: 253–278.
5. Palmiere C. Markers for sepsis diagnosis in the forensic setting: state of the art. *Croat Med J* 2014; 55: 103–114.
6. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998; 102: 1369–1376.
7. Terry CF, Loukaci V and Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. *J Biol Chem* 2000; 275: 18138–18144.
8. Zhou J, Feng J and Li X. Association between the -174G/ C polymorphism of the interleukin-6 gene and myocardial infarction risk: a meta-analysis. *Genet Mol Res* 2016; 15.
9. Lu YM, Cao LF, Li YQ, et al. RANTES gene polymorphisms and risk of pediatric asthma: a meta-analysis. *Exp Ther Med* 2012; 4: 918–922.
10. Higgins JP and Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; 21: 1539–1558.
11. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315: 629–634.
12. Schlüter B, Raufhake C, Erren M, et al. Effect of the interleukin-6 promoter polymorphism (-174G/C) on the incidence and outcome of sepsis. *Crit Care Med* 2002; 30: 32–37.
13. Treszl A, Kocsis I, Szathmári M, et al. Genetic variants of TNF-alpha, IL-1beta, IL-4 receptor alpha-chain, IL-6 and IL-10 genes are not risk factors for sepsis in low-birthweight infants. *Biol Neonate* 2003; 83: 241–245.
14. Balding J, Healy CM, Livingstone WJ, et al. Genomic polymorphic profiles in an Irish population with meningococcaemia: is it possible to predict severity and outcome of disease. *Genes Immun* 2003; 4: 533–540.
15. Ahrens P, Kattner E, Köhler B, et al. Mutations of genes involved in the innate immune system as predictors of sepsis in very low birth weight infants. *Pediatr Res* 2004; 55: 652–656.
16. Sipahi T, Pocan H and Akar N. Effect of various genetic polymorphisms on the incidence and outcome of severe sepsis. *Clin Appl Thromb Hemost* 2006; 12: 47–54.
17. Göpel W, Härtel C, Ahrens P, et al. Interleukin-6-174-genotype, sepsis and cerebral injury in very low birth weight infants. *Genes Immun* 2006;7: 65–68.
18. Baier RJ, Loggins J and Yanamandra K. IL-10, IL-6 and CD14 polymorphisms and sepsis outcome in ventilated very low birth weight infants. *BMC Med* 2006; 4: 10.
19. Michalek J, Svetlikova P, Fedora M, et al. Interleukin-6 gene variants and the risk of sepsis development in children. *Hum Immunol* 2007; 68: 756–760.
20. Abdel-Hady H, El-Naggar M, El-Nady G, et al. Genetic polymorphisms of IL-6-174 and IL-10-1082 in full term neonates with late onset blood stream infections. *J Pediatr Infect Dis* 2009; 4: 357–365.
21. Davis SM, Clark EA, Nelson LT, et al. The association of innate immune response gene polymorphisms and puerperal group A streptococcal sepsis. *Am J Obstet Gynecol* 2010; 202: 308.e1–8.
22. Carregaro F, Carta A, Cordeiro JA, et al. Polymorphisms IL10–819 and TLR-2 are potentially associated with sepsis in Brazilian patients. *Mem Inst Oswaldo Cruz* 2010; 105: 649–656.
23. Shimada T, Oda S, Sadahiro T, et al. Outcome prediction in sepsis combined use of genetic polymorphisms – a study in Japanese population. *Cytokine* 2011; 54: 79–84.
24. Martín-Loeches I, Solé-Violán J, Rodríguez de Castro F, et al. Variants at the promoter of the interleukin-6 gene are associated with severity and outcome of pneumococcal community-acquired pneumonia. *Intensive Care Med* 2012; 38: 256–262.
25. Panayides A, Ioakeimidou A, Karamouzos V, et al. -572G/C single nucleotide polymorphism of interleukin-6 and sepsis predisposition in chronic renal disease. *Eur J Clin Microbiol Infect Dis* 2015; 34: 2439–2446.
26. Mao ZR, Zhang SL and Feng B. Association of IL-10 (-819T/C,-592A/C and -1082A/G) and IL-6-174G/C gene polymorphism and the risk of pneumonia-induced sepsis. *Biomarkers* 2017; 22: 106–112.
27. Gu W, Du DY, Huang J, et al. Identification of interleukin-6 promoter polymorphisms in the Chinese Han population and their functional significance. *Crit Care Med* 2008; 36: 1437–1443.
28. Chérel M, Campion L, Bézieau S, et al. Molecular screening of interleukin-6 gene promoter and influence of -174G/C polymorphism on breast cancer. *Cytokine* 2009; 47: 214–223.