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Association between interleukin-10 polymorphisms and sepsis: a meta-analysis

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SUMMARY

Genetic association studies of the cytokine interleukin-10 (IL-10) and sepsis have provided inconsistent results. This work attempts to further quantitatively assess the association of three widely evaluated polymorphisms of IL-10 (−592C/A, −819C/T, −1082A/G) with sepsis susceptibility through a meta-analysis. A search of Pubmed, Web of Science and EMBASE databases was performed. Overall, the three polymorphisms have no strong association with sepsis risk. Subgroup analysis by ethnicity showed there was association between sepsis susceptibility with −592C/A in Caucasians (A *vs.* C: OR 0·78, 95% CI 0·62–1·00, $P=0·05$; AA + CA *vs.* CC: OR 0·75, 95% CI 0·56–1·00, $P=0·05$), and with −1082A/G in Asians (G *vs.* A: OR 1·41, 95% CI 1·04–1·91, $P=0·03$; GG + AG *vs.* AA: OR 2·11, 95% CI 1·07–4·16, $P=0·03$). This meta-analysis suggests that −592C/A and −1082A/G polymorphisms are associated with sepsis susceptibility in Caucasian, and Asian populations, respectively.

Key words: Genetic polymorphism, interleukin-10, sepsis.

INTRODUCTION

Sepsis is defined as the systemic inflammatory response caused by infection [1]. Despite modern resuscitation strategies and new anti-infective options, sepsis and its sequelae remain a common cause of acute illness and death in patients with community-acquired and nosocomial infections [2]. Therefore, predictive markers to identify high-risk patients are urgently needed for early detection and preventive care. Increasing evidence suggests that genetic variation especially single nucleotide polymorphisms (SNPs) in the innate immune system may influence the risk of patients for serious infection [3]. Delineating the variation in genes and associated differences in response to sepsis may contribute to the development of new genetically tailored diagnostic and therapeutic interventions to improve outcome in patients with sepsis susceptibility.

Interleukin-10 (IL-10) has been identified as one of the key anti-inflammatory cytokines in the inflammatory cascade as it decreases the production of inflammatory molecules, such as TNF-α, interferon (IFN)-γ, IL-12, reactive nitric oxide metabolites, major histocompatibility complex molecules [4] and inhibits antigen-specific cytotoxic T cells [5]. IL-10 has been shown to be elevated after trauma [6] and is well correlated with the development of sepsis and outcome in patients with major trauma [7, 8]. Previous studies have demonstrated that variation in IL-10 production

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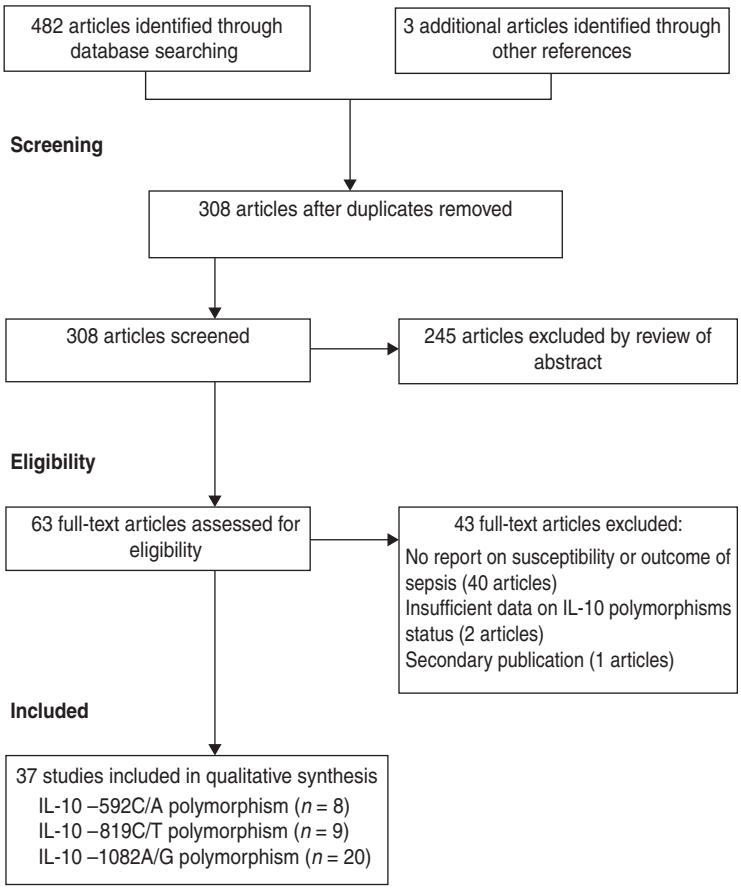

Fig. 1. Flow diagram of study identification and selection.

is largely genetically determined through variations in the promoter region [9, 10]. The IL-10 5′-flanking region, which controls transcription, is polymorphic, with two microsatellites between −4000 and −1100, and three SNPs (−1082, −819, −592) [11]. Moreover, the IL-10 −1082AA genotype is related to lower production of IL-10, and carriers with heterozygous AG genotype and homozygous GG genotype have significantly increased inducibility of IL-10 production after stimulation [12–14].

To date, several studies have investigated the association of IL-10 polymorphisms with sepsis susceptibility but their findings lack consistency and remain inconclusive. Only one meta-analysis has investigated the association between the IL-10 −1082A/G polymorphism and sepsis risk [15]. This paper from 2013, included 11 studies published before September 2012, and concluded that the IL-10 −1082A/G polymorphism has an association with susceptibility to sepsis but the inclusion of overlapping studies [16, 17] might have biased their findings. We performed an initial systematic literature search which revealed several other studies on the −1082A/G polymorphism not cited in [15]. This therefore prompted a meta-analysis to investigate further associations between three widely evaluated variants of IL-10: −592C/A (rs1 800 872), −819C/T (rs1 800 871), and −1082A/G (rs1 800 896), and sepsis susceptibility.

METHODS

Literature search

A systematic literature search for published papers was performed in Pubmed, EMBASE, and Web of Science for the period up to 8 October 2013. The following key terms were used: (interleukin-10 OR IL10 OR IL-10) AND (polymorphism OR mutation OR variant) AND (sepsis OR septic). The references of original research reports and review articles were also searched. Studies fulfilling all of the following selection criteria were included: (1) they evaluated the association between polymorphisms in the IL-10 gene and risk of sepsis; (2) they were case-control

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Table 1. *Characteristics of the studies included in the meta-analysis*

Study	Country	Ethnicity	Sepsis type	Source of controls	Genotyping method	Sample size (cases/controls)	Cases (11/12/22)*	Controls (11/12/22)*	NOS score	HWE
rs1800872 −592C/A										
Shimada, 2011 [21]	Japan	Asian	Sepsis	Critically ill	Fluorescence probe	123/101	62/47/14	38/45/18	6	Yes
Davis, 2010 [22]	America	Caucasian	Sepsis	Healthy	TaqMan probe	26/53	18/8/0	30/17/6	7	Yes
Gu, 2010 [16]	China	Asian	Sepsis	Trauma	Fluorescence probe	153/154	17/66/70	26/57/71	7	No
Huebinger, 2010 [23]	America	Caucasian	Sepsis	Burn	TaqMan probe	50/215	22/24/4	95/98/22	6	Yes
Nakada, 2005 [24]	Japan	Asian	Sepsis	Critically ill	PCR–RFLP	86/111	8/38/40	19/47/45	6	Yes
Balding, 2003 [25]	Ireland	Caucasian	Sepsis	Healthy	PCR–RFLP	183/389	125/52/6	235/139/15	5	Yes
Lowe, 2003 [26]	UK	Caucasian	Sepsis	Critically ill	PCR–RFLP	41/36	30/10/1	23/13/0	6	Yes
Shu, 2003 [27]	China	Asian	Severe sepsis	Healthy	PCR–RFLP	116/141	71/38/7	91/39/11	6	No
rs1800871 −819C/T										
Palumbo, 2012 [28]	Italy	Caucasian	Sepsis	Burn	PCR–RFLP	16/26	10†/6‡	12†/14‡	7	n.a.
Shimada, 2011 [21]	Japan	Asian	Sepsis	Critically ill	Fluorescence probe	123/101	62/47/14	38/46/17	6	Yes
Carregaro, 2010 [29]	Brazil	Caucasian	Sepsis	Blood donor	TaqMan probe	97/207	47/38/12	82/102/23	6	Yes
Davis, 2010 [22]	America	Caucasian	Sepsis	Healthy	TaqMan probe	28/53	18/10/0	30/17/6	7	Yes
Emonts, 2010 [30]	Netherlands	Caucasian	Sepsis	Healthy	SNaPshot	79/460	49/24/6	283/145/32	6	No
Gu, 2010 [16]	China	Asian	Sepsis	Trauma	Fluorescence probe	148/160	12/49/87	17/73/70	7	Yes
Huebinger, 2010 [23]	America	Caucasian	Sepsis	Burn	TaqMan probe	50/215	22/24/4	95/98/22	6	Yes
Nakada, 2005 [24]	Japan	Asian	Sepsis	Critically ill	PCR–RFLP	86/111	8/38/40	19/47/45	6	Yes
Shu, 2003 [27]	China	Asian	Severe sepsis	Healthy	PCR–RFLP	116/141	71/38/7	91/39/11	6	No
rs1800896 −1082A/G										
Palumbo, 2012 [28]	Italy	Caucasian	Sepsis	Burn	PCR–RFLP	16/26	12§/4¶	5§/21¶	7	n.a.
Shimada, 2011 [21]	Japan	Asian	Sepsis	Critically ill	Fluorescence probe	123/101	111/12/0	93/8/0	6	Yes
Carregaro, 2010 [29]	Brazil	Caucasian	Sepsis	Blood donor	TaqMan probe	97/207	44/44/9	82/103/22	6	Yes
Davis, 2010 [22]	America	Caucasian	Sepsis	Healthy	TaqMan probe	28/53	7/16/5	16/25/12	7	Yes
Emonts, 2010 [30]	Netherlands	Caucasian	Sepsis	Healthy	SNaPshot	82/459	17/44/21	117/219/123	6	Yes
Gu, 2010 [16]	China	Asian	Sepsis	Trauma	Fluorescence probe	147/161	106/37/4	118/36/7	7	Yes
Abdel, 2009 [31]	Egypt	Caucasian	Sepsis	Neonates	PCR–RFLP	54/70	11/37/6	14/47/9	5	No
McDaniel, 2007 [32]	America	Caucasian	Sepsis	Trauma	ARMS	31/37	7/13/11	18/11/8/	5	No
Baier study1, 2006 [12]	America	Caucasian	Sepsis	VLBW	ARMS	114/119	48/57/9	40/61/18	6	Yes
Baier study2, 2006 [12]	America	Caucasian	Sepsis	VLBW	ARMS	31/26	13/13/5	6/12/8/	6	Yes
Garnacho, 2006 [33]	Spain	Caucasian	Sepsis	Healthy	PCR–RFLP	224/101	92/99/33	36/50/15	7	Yes
Stanilova, 2006 [34]	Bulgaria	Caucasian	Severe sepsis	Healthy	ARMS	33/53	17/15/1	12/32/9	5	Yes
Nakada, 2005 [24]	Japan	Asian	Sepsis	Critical ill	PCR–RFLP	86/111	75/11/0	103/8/0	6	Yes
Zhang, 2005 [35]	China	Asian	Septic shock	Critical ill	PCR–RFLP	33/76	19/14/0	62/14/0	6	Yes
Jaber, 2004 [36]	America	Caucasian	Sepsis	ARF	SSP–PCR	38/21	13[a]/25[b]	4[a]/17[b]	5	NA

Table 1 (*cont.*)

Study	Country	Ethnicity	Sepsis type	Source of controls	Genotyping method	Sample size (cases/controls)	Cases (11/12/22)*	Controls (11/12/22)*	NOS score	HWE
Balding, 2003 [25]	Ireland	Caucasian	Sepsis	Healthy	PCR–RFLP	183/389	36/95/52	86/180/123	5	Yes
Lowe, 2003 [26]	UK	Caucasian	Sepsis	Critical ill	PCR–RFLP	31/36	8/18/5	11/17/8	6	Yes
Schaaf, 2003 [13]	Germany	Caucasian	Sepsis	Infection	ARMS	51/18	17/20/14	8/8/2/	6	Yes
Shu, 2003 [27]	China	Asian	Severe sepsis	Healthy	PCR–RFLP	116/141	8/77/31	42/56/43	6	No
Treszl, 2003 [37]	Hungary	Caucasian	Sepsis	VLBW	PCR–RFLP	33/70	10/17/6	11/40/19	6	Yes

ARF, Acute renal failure; ARMS: amplification refractory mutation system; HWE, Hardy–Weinberg equilibrium; n.a., not available; NOS, Newcastle–Ottawa Scale; PCR, polymerase chain reaction; PCR–RFLP, PCR–restriction fragment length polymorphism; SSP–PCR, single-stranded peptide–PCR; VLBW, very-low-birth-weight neonates.
* For −592C/A polymorphism (11 = CC, 12 = CA, 22 = AA); for −819C/T polymorphism (11 = CC, 12 = CT, 22 = TT); for −1082A/G polymorphism (11 = AA, 12 = AG, 22 = GG).
† Genotype represented by 11.
‡ Genotype represented by 12 + 22.
§ Genotype represented by 11 + 12.
¶ Genotype represented by 22.

or cohort studies; (3) they provided data on the genotype or allele counts of at least one polymorphism. Studies were excluded if any of the following features were identified: (1) they were not relevant to IL-10 polymorphisms and sepsis; (2) they were non-clinical; (3) they were reviews or comments. Only English-language publications were included. For overlapping studies, the one with the largest sample size was included.

Qualitative assessment and data extraction

Two investigators (W.P. and A.Q.Z.) independently extracted data, and made a quality assessment of the retrieved studies. Discrepancies were resolved in a consensus meeting. The Newcastle–Ottawa Scale (NOS) [18] was applied to evaluate the qualities of the included studies. A scoring system of 0–9 was used to judge and score data study quality based on three broad perspectives: selection, comparability, and outcome or finding of interest. Studies with scores of $\geqslant 7$ were considered to be of high quality and scores were summed to quantitatively compare the study quality.

The following information was extracted from each study: authors, year of publication, country, ethnicity of participants, source of controls, sample size, genotype distribution, sepsis type and genotyping method.

Statistical analysis

Data were entered from studies into a computerized spreadsheet (Microsoft Excel, USA). Hardy–Weinberg equilibrium (HWE) – to measure whether the observed genotype frequencies in a population differ from the frequencies predicted by the equation – was tested by means of χ^2 test (significant at the 0·05 level). The odds ratios (ORs) and 95% confidence intervals (CIs) as the effect measure were estimated for each study. The significance of pooled ORs was tested by Z test ($P < 0{\cdot}05$) and pooled ORs were evaluated for the allele comparison model (B *vs.* A), the co-dominant model (BB *vs.* AA), the dominant model (BB + AB *vs.* AA), and the recessive model (BB *vs.* AA + AB); A and B represent the major and minor alleles, respectively. As heterogeneity was anticipated between observational studies, pooled ORs were calculated using a random-effects model for all analyses. Consistent with common practice, we performed a statistical test for heterogeneity between studies using Cochran's Q; a significant Q statistic ($P < 0{\cdot}10$) indicated heterogeneity across studies. The I^2 statistic was used for estimation of the degree

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Table 2. *Overall and subgroup analyses of the IL-10 −592C/A polymorphism with sepsis susceptibility*

		Sample size		Test of association			Heterogeneity	
Groups	Studies	Cases	Controls	*P* value	OR (95% CI)	Model	*P* value	I^2 (%)
Total								
A *vs.* C	8	778	1200	0·36	0·91 (0·74–1·11)	R	0·12	39
AA *vs.* CC	8	778	1200	0·79	0·94 (0·60–1·48)	R	0·20	29
AA *vs.* CC+CA	8	778	1200	0·56	0·92 (0·70–1·22)	R	0·66	0
AA+CA *vs.* CC	8	778	1200	0·61	0·92 (0·69–1·25)	R	0·09	44
Asian								
A *vs.* C	4	478	507	0·95	1·01 (0·75–1·36)	R	0·07	57
AA *vs.* CC	4	478	507	0·87	1·06 (0·55–2·03)	R	0·07	58
AA *vs.* CC+CA	4	478	507	0·76	0·95 (0·70–1·29)	R	0·43	0
AA+CA *vs.* CC	4	478	507	0·59	1·15 (0·69–1·94)	R	0·04	64
Caucasian								
A *vs.* C	4	300	693	0·05	0·78 (0·62–1·00)	R	0·59	0
AA *vs.* CC	4	300	693	0·38	0·73 (0·36–1·47)	R	0·59	0
AA *vs.* CC+CA	4	300	693	0·47	0·77 (0·39–1·54)	R	0·57	0
AA+CA *vs.* CC	4	300	693	0·05	0·75 (0·56–1·00)	R	0·73	0
HWE								
A *vs.* C	6	509	905	0·18	0·83 (0·64–1·08)	R	0·12	43
AA *vs.* CC	6	509	905	0·55	0·83 (0·44–1·54)	R	0·17	35
AA *vs.* CC+CA	6	509	905	0·60	0·90 (0·62–1·32)	R	0·44	0
AA+CA *vs.* CC	6	509	905	0·13	0·79 (0·58–1·07)	R	0·23	27
Sepsis								
A *vs.* C	7	662	1059	0·31	0·89 (0·70–1·12)	R	0·08	46
AA *vs.* CC	7	662	1059	0·87	0·96 (0·56–1·63)	R	0·14	38
AA *vs.* CC+CA	7	662	1059	0·56	0·94 (0·70–1·25)	R	0·56	0
AA+CA *vs.* CC	7	662	1059	0·50	0·89 (0·63–1·25)	R	0·08	47

OR, Odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

of heterogeneity in meta-analyses and a study with an I^2 of >50% was interpreted as having a high degree of heterogeneity [19]. In addition, several factors, such as ethnicity, age, and sepsis severity, were closely related to susceptibility of sepsis, thus subgroup analyses were performed by ethnicity, sepsis type and HWE. Sensitivity analysis was performed through sequentially excluded individual studies to assess the stability of the results. Potential publication bias was examined visually in a funnel plot of log (OR) against its standard error (s.e.), and the degree of asymmetry was tested using Egger's test [20]. All statistical tests were performed using Revman 5.2 software (Nordic Cochrane Center, Denmark) and Stata v. 11.0 software (Stata Corporation, USA).

RESULTS

Study characteristics

The search of databases retrieved 482 articles and three additional articles were identified from the reference lists of relevant studies giving a total of 485 relevant titles and abstracts for review. After removing 177 duplications, a further unrelated 245 articles were excluded based on title and abstract. After a full text review we excluded a further 43, leaving 37 studies for inclusion in the meta-analysis (Fig. 1).

The main characteristics of the 37 eligible studies and genotype distributions of the three polymorphisms (−592C/A, −819C/T, −1082A/G) under study are listed in Table 1. Twenty-four studies were performed in Caucasian populations and 13 in Asian populations. Genotypes were determined by various methodologies: polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) ($n=16$), amplification refractory mutation system (ARMS) ($n=5$), TaqMan probes ($n=7$), SNaPshot ($n=2$), fluorescence-labelled probes ($n=6$), and single-stranded peptide–PCR (SSP–PCR) ($n=1$). The quality scores of most studies ranged from 5 to 7, suggesting moderate quality.

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Table 3. *Overall and subgroup analyses of the IL-10 −819C/T polymorphism with sepsis susceptibility*

		Sample size		Test of association			Heterogeneity	
Groups	Studies	Cases	Controls	*P* value	OR (95% CI)	Model	*P* value	I^2 (%)
Total								
T *vs.* C	8	727	1448	0·95	0·99 (0·80–1·23)	R	0·04	52
TT *vs.* CC	8	727	1448	0·97	0·99 (0·67–1·48)	R	0·22	26
TT *vs.* CC + CT	8	727	1448	0·59	1·09 (0·78–1·53)	R	0·19	29
TT + CT *vs.* CC	9	743	1474	0·48	0·92 (0·73–1·16)	R	0·33	12
Asian								
T *vs.* C	4	473	513	0·61	1·10 (0·76–1·60)	R	0·01	72
TT *vs.* CC	4	473	513	0·77	1·11 (0·56–2·18)	R	0·07	58
TT *vs.* CC + CT	4	473	513	0·61	1·14 (0·69–1·85)	R	0·08	56
TT + CT *vs.* CC	4	473	513	0·74	1·09 (0·66–1·78)	R	0·07	57
Caucasian								
T *vs.* C	4	254	935	0·31	0·89 (0·71–1·11)	R	0·65	0
TT *vs.* CC	4	254	935	0·62	0·88 (0·52–1·48)	R	0·58	0
TT *vs.* CC + CT	4	254	935	0·90	0·97 (0·59–1·60)	R	0·51	0
TT + CT *vs.* CC	5	270	961	0·23	0·84 (0·64–1·12)	R	0·84	0
HWE								
T *vs.* C	6	532	847	0·86	0·97 (0·72–1·32)	R	0·01	65
TT *vs.* CC	6	532	847	0·98	0·99 (0·57–1·73)	R	0·10	46
TT *vs.* CC + CT	6	532	847	0·65	1·10 (0·72–1·69)	R	0·11	44
TT + CT *vs.* CC	6	532	847	0·52	0·89 (0·64–1·26)	R	0·17	36
Sepsis								
T *vs.* C	7	611	1307	0·89	0·98 (0·76–1·26)	R	0·03	58
TT *vs.* CC	7	611	1307	0·95	1·02 (0·64–1·61)	R	0·16	35
TT *vs.* CC + CT	7	611	1307	0·51	1·13 (0·79–1·62)	R	0·17	34
TT + CT *vs.* CC	8	627	1333	0·34	0·88 (0·69–1·14)	R	0·32	14

OR, Odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

Quantitative data synthesis

For the IL-10 −592C/A polymorphism, eight studies encompassing 778 cases and 1200 controls were identified [16, 21–27]. Overall, there was no significant association between this polymorphism and sepsis risk (A *vs.* C: OR 0·91, 95% CI 0·74–1·11, *P* = 0·36; AA *vs.* CC: OR 0·94, 95% CI 0·60–1·48, *P* = 0·79; AA *vs.* CC + CA: OR 0·92, 95% CI 0·70–1·22, *P* = 0·56; AA + CA *vs.* CC: OR 0·92, 95% CI 0·69–1·25, *P* = 0·61) (Table 2). In subgroup analysis, the results showed significant association in Caucasians under two comparison models (A *vs.* C: OR 0·78, 95% CI 0·62–1·00, *P* = 0·05; AA + CA *vs.* CC: OR 0·75, 95% CI 0·56–1·00, *P* = 0·05) (Table 2). However, no significant association was found in subgroup analyses based on sepsis type and HWE under all genetic models.

For the IL-10 −819C/T polymorphism, nine studies comprised 743 cases and 1474 controls [16, 21–24, 27–30]. Overall, no evidence of a statistically significant association of this polymorphism with sepsis susceptibility was found (T *vs.* C: OR 0·99, 95% CI 0·80–1·23, *P* = 0·95; TT *vs.* CC: OR 0·99, 95% CI 0·67–1·48, *P* = 0·97; TT *vs.* CC + CT: OR 1·09, 95% CI 0·78–1·53, *P* = 0·59; TT + CT *vs.* CC: OR 0·92, 95% CI 0·73–1·16, *P* = 0·48) (Table 3). Similarly, subgroup analyses by ethnicity, sepsis type and HWE revealed no significant association under all genetic models.

For the IL-10 −1082A/G polymorphism, 20 studies of 1551 cases and 2275 controls were identified [12, 13, 16, 21, 22, 24–37]. The overall result suggested no statistically significant association of this polymorphism with sepsis risk (G *vs.* A: OR 1·13, 95% CI 0·89–1·44, *P* = 0·31; GG *vs.* AA: OR 0·91, 95% CI 0·61–1·35, *P* = 0·64; GG *vs.* AG + AA: OR 0·83, 95% CI 0·68–1·02, *P* = 0·08; GG + AG *vs.* AA: OR 1·10, 95% CI 0·82–1·47, *P* = 0·52) (Table 4). However, by subgroup analysis based on ethnicity, there was a statistically

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Table 4. *Overall and subgroup analyses of the IL-10 −1082A/G polymorphism with sepsis susceptibility*

		Sample size		Test of association			Heterogeneity	
Groups	Studies	Cases	Controls	*P* value	OR (95% CI)	Model	*P* value	I^2 (%)
Total								
G *vs.* A	18	1497	2228	0·31	1·13 (0·89–1·44)	R	<0·00001	76
GG *vs.* AA	18	1497	2228	0·64	0·91 (0·61–1·35)	R	0·008	53
GG *vs.* AA + AG	19	1513	2254	0·08	0·83 (0·68–1·02)	R	0·65	0
GG + AG *vs.* AA	19	1535	2249	0·52	1·10 (0·82–1·47)	R	<0·0001	65
Asian								
G *vs.* A	5	354	468	0·03	1·41 (1·04–1·91)	R	0·25	25
GG *vs.* AA	5	354	468	0·58	1·64 (0·29–9·41)	R	0·02	81
GG *vs.* AA + AG	5	354	468	0·36	0·79 (0·48–1·31)	R	0·67	0
GG + AG *vs.* AA	5	354	468	0·03	2·11 (1·07–4·16)	R	0·006	73
Caucasian								
G *vs.* A	13	1143	1760	0·82	1·03 (0·77–1·39)	R	<0·00001	81
GG *vs.* AA	13	1143	1760	0·31	0·82 (0·57–1·19)	R	0·07	39
GG *vs.* AA + AG	14	1159	1786	0·15	0·85 (0·69–1·06)	R	0·56	0
GG + AG *vs.* AA	14	1181	1781	0·34	0·88 (0·67–1·15)	R	0·04	44
HWE								
G *vs.* A	15	1296	1980	0·6	1·08 (0·82–1·42)	R	<0·00001	78
GG *vs.* AA	15	1296	1980	0·10	0·75 (0·54–1·06)	R	0·19	26
GG *vs.* AA + AG	15	1296	1980	0·06	0·80 (0·64–1·01)	R	0·64	0
GG + AG *vs.* AA	15	1296	1980	0·84	0·97 (0·75–1·26)	R	0·02	48
Sepsis								
G *vs.* A	15	1315	1958	0·35	1·13 (0·88–1·46)	R	<0·00001	75
GG *vs.* AA	15	1315	1958	0·36	0·87 (0·63–1·18)	R	0·23	21
GG *vs.* AA + AG	16	1331	1984	0·17	0·86 (0·69–1·07)	R	0·75	0
GG + AG *vs.* AA	16	1353	1979	0·92	0·99 (0·81–1·21)	R	0·21	22

OR, Odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium.

significant association in Asians under two comparison models (G *vs.* A: OR 1·41, 95% CI 1·04–1·91, $P = 0·03$; GG + AG *vs.* AA: OR 2·11, 95% CI 1·07–4·16, $P = 0·03$) (Table 4). No similar association was evident under all comparison models in other subgroup analyses based on sepsis type and HWE.

Heterogeneity analysis

For the −592C/A polymorphism, heterogeneity was significant in the subgroup analyses of Asians and sepsis type. Since the genotype data for two studies were not in HWE [16, 27], a subgroup analysis was performed based on this equation. The exclusion of these latter studies rendered the data more homogeneous (Table 2).

For the −819C/T polymorphism, statistically significant heterogeneity between studies was found in the pooling analyses of total available studies and in the subgroup analyses of Asians and sepsis type. However, results were similar after excluding studies which were not in HWE [27, 28, 30], (Table 3).

For the −1082A/G polymorphism, a significant level of heterogeneity between studies was observed in the overall analyses. Some of the heterogeneity was resolved by ethnicity-specific analyses. Subgroup analyses by HWE and sepsis type were used to pool the data and the results became more homogeneous (Table 4).

Sensitivity analysis

Sensitivity analysis was performed by excluding one study in the meta-analysis at a time to reflect the influence of the individual dataset to the pooled ORs for each of the studied polymorphisms. The corresponding pooled ORs were not significantly altered, which suggested the robustness of the results (data not shown).

Publication bias

Funnel plots and Egger's test were performed to estimate publication bias of the literature. The shapes of

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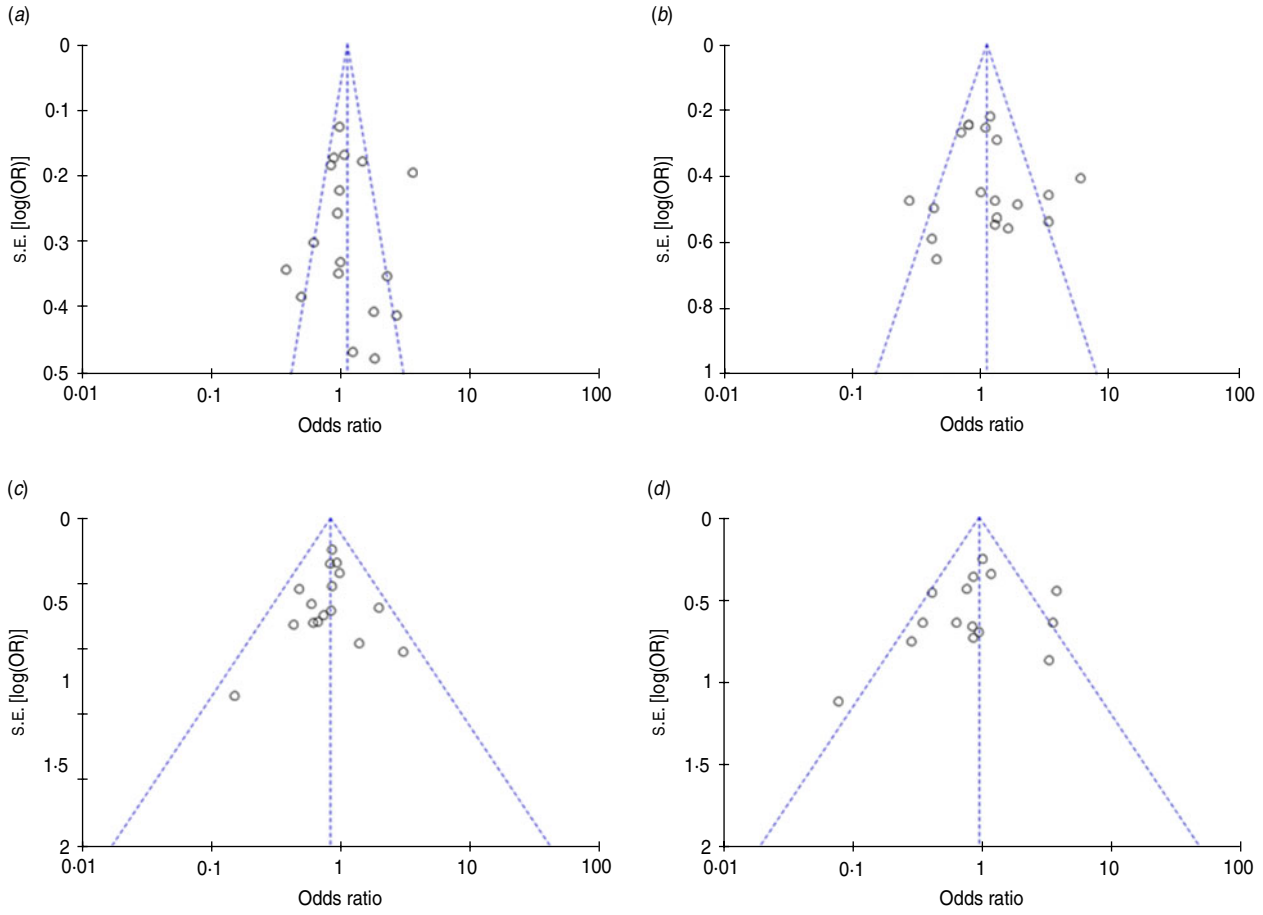

Fig. 2. Funnel plot for IL-10 −1082A/G polymorphism and sepsis risk under the (*a*) G *vs.* A model, (*b*) GG + AG *vs.* AA model, (*c*) GG *vs.* AG + AA model, (*d*) GG *vs.* AA model.

the funnel plots appeared slightly asymmetrical (Fig. 2) but Egger's test revealed no publication bias for studies published on the −1082A/G polymorphism (G *vs.* A: $P = 0{\cdot}939$; GG *vs.* AA: $P = 0{\cdot}096$; GG *vs.* AG + AA: $P = 0{\cdot}456$; GG + AG *vs.* AA: $P = 0{\cdot}754$). Due to the limitation of smaller study sizes, funnel plots and Egger's test were not performed for −592C/A and −819C/T polymorphisms.

DISCUSSION

In this meta-analysis, we found that the pooled ORs and 95% CIs under different comparison models did not show any associations of the A allele of the −592C/A polymorphism, T allele of −819C/T polymorphism and G allele of −1082A/G polymorphism with susceptibility to sepsis in the overall study population. However, subgroup analysis by ethnicity indicated a statistical association between sepsis susceptibility and the −592C/A polymorphism in Caucasian, and the −1082A/G polymorphism in Asian populations. It therefore appears that there is a race-specific effect in the association between the two polymorphisms and sepsis susceptibility in different ethnicities. However, it should be noted that there were only four eligible studies for −592C/A and five studies for −1082A/G available in the current meta-analysis and such small samples with a limited number of subjects may be open to selection bias, and do not categorically support or deny an association. It is therefore critical that larger and well-designed studies are performed to re-evaluate the apparent associations with ethnicity.

Previously, only one meta-analysis has investigated the association between the IL-10 −1082A/G polymorphism and risk of sepsis [15]. This included 11 studies and concluded that the IL-10 −1082A/G polymorphism has an association with susceptibility of sepsis (for G *vs.* A: OR 0·83; for GG *vs.* AA: OR 0·67). However, their findings could be heavily biased as a consequence of the methods used. The inclusion

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of overlapping studies [16, 17] which used the same sample could lead to an underestimation of an association. Moreover, this meta-analysis [15] appeared to lack a robust quality assessment and quantitative data analysis as quality of studies was assessed by HWE in controls and a fixed-effects model (Mantel–Haenszel method) was used in quantitative data analysis. Last, several studies of IL-10 −1082A/G were not included in the analysis [21–23, 25, 30–32, 36, 37], and since their publication a new study of IL-10 gene polymorphisms in burn sepsis has appeared [28].

Statistical heterogeneity between studies is common in meta-analysis of genetic association studies (GAS) and it can be taken into account by performing a random-effects model [38]. In addition, deviations from HWE in control subjects, which can be due to genotyping errors, population stratification, selection bias in the choice of controls and confounding factors unaccounted for, may bias the estimates of genetic effects in GAS and meta-analysis [39], thus we performed subgroup analysis by HWE. No consensus is achieved currently for whether or not to include the studies departing from HWE. But if the results are different before and after removing studies not in HWE, it is suggested that the analysis without studies not conforming to HWE would be more valid [40].

Our meta-analysis has several limitations. The number of included studies was relatively small for the stratified analysis and low sample size could also introduce bias into the analysis of ethnic populations, sepsis types and HWE. Further, we did not address the effect of gene–gene and gene–environment interactions due to a lack of the related information in the analysis and finally, our results were based on unadjusted genotype data. A more precise analysis should be made by adjusting other potentially confounding factors such as age, sex, and environmental factors. Nevertheless, to our knowledge, this is the first comprehensive study that has quantitatively synthesized the association between the three common polymorphisms of IL-10: −592C/A, −819C/T, −1082A/G and sepsis. We suggest that such an approach of combining the results of association studies may help us to better understand the effect of polymorphisms on disease risk.

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DECLARATION OF INTEREST

None.

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