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ORIGINAL PAPER

Associations between PTPN22 and TLR9 polymorphisms and systemic lupus erythematosus: a comprehensive meta-analysis

Li-Ya Hu[1] · Zhi Cheng[2] · Bo Zhang[3] · Qiong Yin[1] · Xiao-Wei Zhu[1] · Pian-Pian Zhao[1] · Ming-Yu Han[4] · Xiao-Bo Wang[1] · Hou-Feng Zheng[1,5]

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Abstract Previous studies have explored the relationship of PTPN22 and TLR9 polymorphisms with systemic lupus erythematosus (SLE). In consideration of the population stratification, conflicting results and updating data, we conducted a comprehensive meta-analysis, which consists of a total of 17 research articles (9120 cases and 11,724 controls) for PTPN22 and 20 articles (including up to 2808 cases and 3386 controls) for TLR9. Significant association was verified between PTPN22 rs2476601 and SLE in the overall population (OR = 1.511 per T allele, 95% CI 1.338–1.706, $P = 2.931 \times 10^{-11}$) and under dominant model of T allele (TT+CT vs. CC: OR = 1.531, 95% CI 1.346–1.742, $P = 9.17 \times 10^{-11}$). Analysis after stratification by ethnicity indicated that PTPN22 rs2476601 was related to SLE in Americans (OR = 2.566, 95% CI 1.796–3.665, $P = 2.219 \times 10^{-7}$), Europeans (OR = 1.399, 95% CI 1.261–1.552, $P = 2.153 \times 10^{-10}$), and Africans (OR = 4.14, 95% CI 1.753–9.775, $P = 1.0 \times 10^{-3}$). We did not observe any association between TLR9 polymorphisms (rs187084, rs352140, rs5743836 and rs352139) and SLE under any model, after excluding the data that were inconsistent with Hardy–Weinberg equilibrium (HWE). In summary, PTPN22 rs2476601 was significantly interrelated with SLE and contributed to susceptibility and development of SLE in Americans, Europeans and Africans in this analysis, while their relationship needs to be validated in Africans by future research.

Keywords Protein tyrosine phosphatase non-receptor 22 · Toll-like receptor 9 · Polymorphism · Systemic lupus erythematosus · Meta-analysis

Li-Ya Hu, Zhi Cheng and Bo Zhang contributed equally to this work.

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✉ Hou-Feng Zheng
h-zheng@live.com

[1] Institute of Aging Research and the Affiliated Hospital, School of Medicine, Hangzhou Normal University, Hangzhou, Zhejiang, China

[2] Suzhou Science and Technology Town Hospital, Suzhou Hospital Affiliated to Nanjing Medical University, Suzhou, Jiangsu, China

[3] The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China

[4] School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland

[5] Institute for Basic Medical Sciences, Westlake Institute for Advanced Study, Hangzhou, Zhejiang, China

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease typically characterized by the generation of multiple autoantibodies to nuclear antigens, and by complement activation and immune complex deposition, which results in diverse clinical manifestation and the involvement of multisystem [85]. It has been confirmed that genetic and environmental factors play important role in the development of SLE [27, 34, 85]. The morbidity rate of SLE is diversiform in different populations [39], with 31–70/100,000 in Chinese [88], and 7–71/100,000 in European [12] and up to 200/100,000 in African populations [12]. Although, abundant studies have been carried out, the etiology and pathogenesis of SLE have not been clearly illustrated. Genome-wide linkage studies have

evidenced susceptibility area in SLE, such as 1q23 [80], 4p16-15.2 [24], 11p13 [53], 11q14 [73], 12q24 [54], 16p13 [90] and 16q12 [80], and dozens of valuable susceptible genes have been found, such as IKBKE [72], IL8 [72], MAMDC1 [28], PTPN22 [78], STAT4 [62], FCGR2B [95], FCGR3B [95] and TLR5 [70]. Recently, genome-wide association studies (GWAS) have provided more clues for the studies of complex diseases, which assisted us in discovering the susceptibility genes and SNPs for SLE [26, 49, 56, 86].

PTPN22 (protein tyrosine phosphatase non-receptor 22) gene locates on chromosome 1p13.3-p13.1 and encodes lymphoid-specific phosphatase (Lyp) [67]. Lyp is known to participate in antigen receptor signaling and its impact on the immune response and role in the development of autoimmunity have been a focus of current research [67]. Lyp inhibits T cell activation by binding to C-terminal Src tyrosine kinase (Csk). PTPN22 participates in the inhibition of T cell activation, inactivates kinase of T cell receptor and limits to the reaction to the antigen [10]. The mutation of PTPN22 rs2476601 single-nucleotide polymorphism (SNP) could disrupt the physiological interaction between Lyp gene and Csk kinase, and cause signal transduction disorder and reduce the threshold for T cell receptor signaling, leading to the occurrence of autoimmune diseases [11, 82], including SLE, rheumatoid arthritis, type 1 diabetes and autoimmune thyroid disease [9]. To date, the GWAS have reported the association between PTPN22 rs2476601 and autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis, myasthenia gravis and Crohn's disease [3, 22, 25, 58]. The GWAS have also confirmed the significant association between PTPN22 rs2476601 and SLE in Europeans [6, 49]. Although results from GWAS were sometimes taken as the golden criterion for the association study, conflicting findings still existed in different studies, which might have resulted from greater variability of allele frequencies in different populations.

Toll-like receptor (TLR) family is a transmembrane pattern recognition receptor, which plays an important part in the activation and regulation of both innate and adaptive immune response. TLR9 is a kind of specific receptor, which can recognize cytosine phosphate guanosine (CpG) located in bacterial DNA [29]. The aberrant activation of TLR9 may lead to the production of type 1 interferon, which has been closely related to SLE pathophysiology [27]. Study has confirmed that TLR9 plays an important role in the generation and development of SLE by combining with the CpG motif [37].

Migita et al. [47] showed that TLR9 expression in lymphocytes was increased in patients with SLE. However, Hur et al. [33] observed that four polymorphisms within a 4334-bp segment of the TLR9 gene demonstrated no significant association with susceptibility to SLE in a Korean population. De Jager et al. [13] examined a much larger 68,742-bp segment that contains the entire TLR9 gene and 60 kb of flanking sequence, and no evidence of association was found.

Although a large number of studies have investigated the associations between PTPN22 SNPs (rs2476601), TLR9 SNPs (rs187084, rs352140, rs5743836, rs352139) and SLE risk, the results were inadequate or discordant. To check the association in different ethnics, we performed a comprehensive ethnicity-specific meta-analysis to illuminate the association of PTPN22 and TLR9 with SLE susceptibility by enhancing statistical power. We also tried to find the best-fit association model among the dominant, recessive and additive models for the polymorphisms.

Methods

Identification of eligible studies

A comprehensive literature research was conducted by the PubMed databases (National Center for Biotechnology, National Library of Medicine), CNKI (Chinese National Knowledge Infrastructure) and Wanfang databases (Wanfang Data Knowledge Service Platform) for the association between PTPN22 or TLR9 polymorphisms with SLE before December 2016, with the following key words: 'The protein tyrosine phosphatase non-receptor 22', 'PTPN22', 'Toll-like receptor 9', 'TLR9', 'polymorphism', 'Systemic lupus erythematosus' and 'SLE'.

Inclusion and exclusion criteria

The included study should satisfy the following criteria: (1) case–control study; (2) providing raw data, from which the odds ratio (OR) and 95% confidence interval (CI) could be calculated; (3) all patients should fulfil the American College of Rheumatology (ACR) criteria [31]. Exclusion criteria were as follows: (1) data from conference or meeting abstracts; (2) family-based study; (3) study without genotype distributions.

Data extraction

The following information is extracted from each study: journal, first author, year of publication, ethnicity, the sample size, genotype distributions and the allele frequency of cases and controls. We calculated the number of genotype by allele frequency with Hardy–Weinberg equilibrium (HWE) law, when the data was insufficient in the articles.

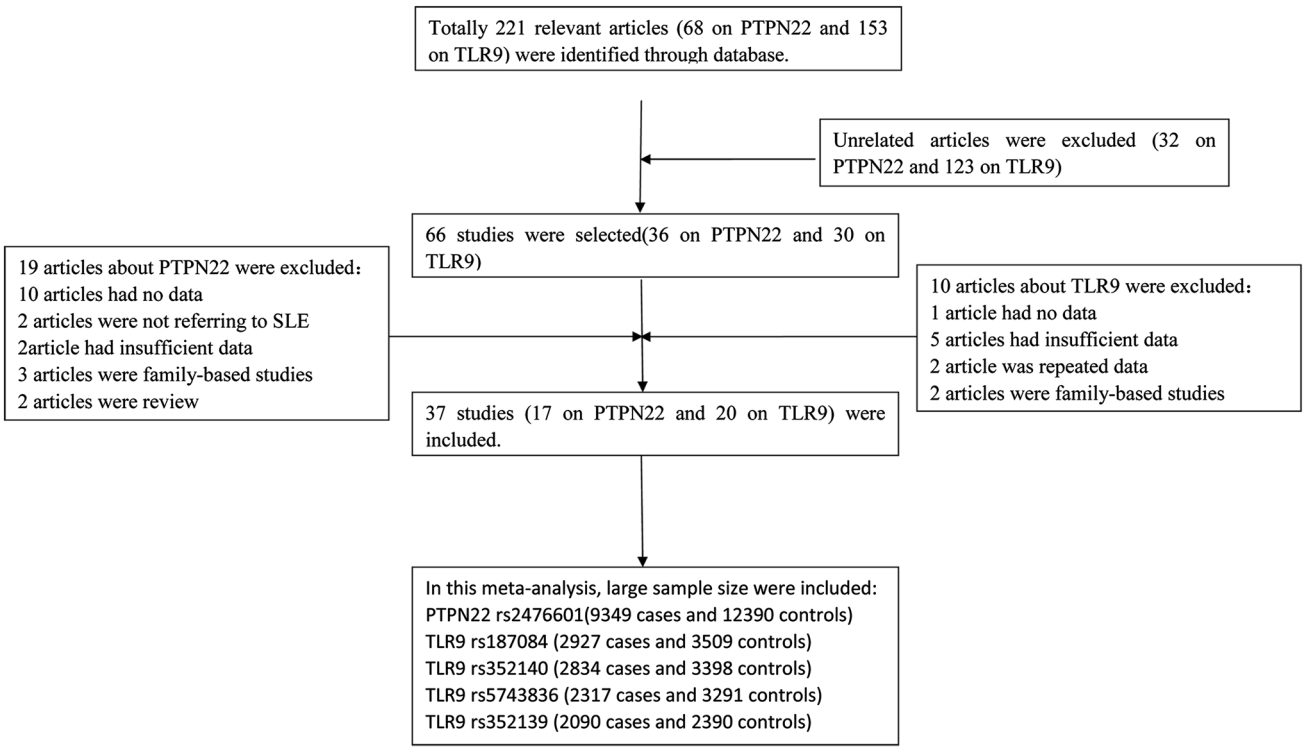

Fig. 1 Flow diagram of the included and excluded articles in this meta-analysis

Statistical analyses

We calculated the Hardy–Weinberg equilibrium (HWE) of control group separately in each of the study, and we considered that the study population did not conform to HWE when the P value for HWE <0.05. Allele frequencies of PTPN22 and TLR9 polymorphisms from each study were determined by allele counting. OR and 95% CIs were calculated to evaluate the association between PTPN22 SNP or TLR9 SNPs and SLE; meanwhile, Cochran's Q-statistic was used to estimate the effect of variations and heterogeneities [30]. If a significant Q-statistic indicated heterogeneity across studies ($P < 0.10$), then the random effect model was applied to the meta-analysis; otherwise, the fixed effect model was used [15, 30]. The effect of heterogeneity was quantified with the following formula: $I^2 = 100\% \times (Q - df)/Q$ [30]. The I^2 value represented the proportion of between-study variability attributable to heterogeneity, and I^2 values of 25, 50 and 75% were considered to be of low, moderate and high heterogeneity, respectively. If study groups showed no heterogeneity, fixed and random effects models produced similar results and, if not, the random effects model usually produced wider CIs than the fixed effects model. Thus, random effects model was applied to this analysis of significant between-study heterogeneity.

The potential publication bias was evaluated using Begg's linear regression test [4], which measured the funnel plot asymmetry using a natural logarithm scale of ORs. A sensitivity analysis was performed to assess the influence of each study on the total effect. The statistical analysis was carried out using STATA 12.0 (Stata Corporation, College Station, TX, USA).

Results

Studies included in the meta-analysis

In this meta-analysis, totally 221 relevant articles (68 for PTPN22 and 153 for TLR9) were found. After reading titles and abstracts, we selected 66 articles (36 for PTPN22 and 30 for TLR9) for further reading. Then, we excluded 29 articles (19 for PTPN22 and 10 for TLR9), because of no data, insufficient data, repeated date, family-based studies, not referring to SLE or reviews. Thus, 37 articles (17 for PTPN22 and 20 for TLR9) met the study inclusion criteria [1, 2, 14, 16, 18–21, 23, 32, 33, 35, 38, 45, 46, 48, 52, 55, 57, 59–61, 63–66, 68, 69, 74, 76, 77, 79, 82, 84, 89, 93, 94]. The flowchart of selecting article process is presented in Fig. 1. Totally, there were 9349 cases and 12,390 controls to evaluate the relationship between PTPN22 rs2476601 and SLE. For TLR9, there were 2927 cases and

Table 1 Characteristics of the (a) PTPN22 and (b) TLR9 polymorphism allelic and genotype distribution among SLE patients and control subjects

(a) PTPN22

Polymorphisms and study	Journals	Years	Ethnicity	Sample Size		Genotypes						Allele frequencies (%)				HWE
				Case	Control	Case			Control			Case		Control		
SNP rs2476601						TT	CT	CC	TT	CT	CC	T	C	T	C	*P*
Baca et al. [2]	Genes Immunity	2006	American	250	355	0	17	233	0	8	347	0.034	0.966	0.011	0.989	1.000
Orozco et al. [57]	Arthritis Rheum	2005	European	338	512	2	62	274	4	63	445	0.098	0.902	0.069	0.931	0.2915
Moez and Soliman [48]	Rheumatol Int	2012	African	40	20	0	21	19	0	4	16	0.263	0.737	0.1	0.9	1.000
Eliopoulos et al. [20]	Lupus	2011	European	328	427	0	33	295	0	23	404	0.05	0.95	0.027	0.973	1.000
Machado Contreras et al. [46]	Clin Exp Med	2015	American	150	150	0	9	141	0	5	145	0.03	0.97	0.017	0.983	1.000
Elghzaly et al. [19]	Human Immunol	2015	African	170	241	0	10	160	0	3	283	0.029	0.971	0.005	0.995	1.000
Aksoy et al. [1]	Mol Biol Rep	2011	Asian	137	160	0	8	129	0	7	153	0.029	0.971	0.022	0.978	1.000
Ostanek et al. [59]	Mol Biol Rep	2014	European	135	201	5	49	81	3	50	148	0.219	0.781	0.139	0.861	0.7731
Gomez et al. [23]	Genes Immunity	2005	American	143	308	3	24	116	0	27	281	0.105	0.895	0.044	0.956	1.000
Reddy et al. [68]	Genes Immunity	2005	European	571	1042	19	151	401	21	214	807	0.165	0.835	0.123	0.877	0.1484
Peng and Ou [61]	J South Med Univ	2010	Asian	40	20	1	3	36	0	2	18	0.063	0.937	0.05	0.95	1.000
Viken et al. [82]	Genes Immunity	2010	European	162	555	2	31	129	7	114	434	0.108	0.892	0.115	0.885	1.000
Kaufman et al. [35]	Arthritis Rheum	2006	European	279	740	2	42	235	9	108	623	0.082	0.918	0.085	0.915	0.0949
Kaufman et al. [35]	Arthritis Rheum	2006	African	131	494	0	7	124	3	16	475	0.027	0.973	0.022	0.978	0.001
Kaufman et al. [35]	Arthritis Rheum	2006	European	98	172	0	19	79	3	11	158	0.097	0.903	0.049	0.951	0.004
Kyogoku et al. [38]	Am J Hum Genet	2004	European	365	926	14	67	284	9	143	774	0.13	0.87	0.087	0.913	0.4046
Kyogoku et al. [38]	Am J Hum Genet	2004	European	340	1035	7	69	264	3	172	860	0.122	0.878	0.086	0.914	0.0737
Namjou et al. [52]	PLoS One	2013	European	3936	3491	47	764	3125	22	514	2955	0.109	0.891	0.08	0.92	1.000
Namjou et al. [52]	PLoS One	2013	European	1492	807	4	153	1335	2	69	736	0.054	0.946	0.045	0.955	0.6752
Ramirez et al. [66]	Clin Exp Rheumatol	2012	American	94	434	1	12	81	1	24	409	0.074	0.926	0.03	0.97	0.3201
Piotrowski et al. [64]	Clin Exp Rheumatol	2008	European	150	300	5	54	91	4	69	227	0.213	0.787	0.128	0.872	0.7987

(b) TLR9

Polymorphisms and study	Journals	Years	Ethnicity	Sample Size		Genotypes						Allele frequencies (%)				HWE
				Case	Control	Case			Control			Case		Control		
SNP rs187084						CC	CT	TT	CC	CT	TT	C	T	C	T	*P*
Huang et al. [32]	Rheumatol Int	2012	Asian	167	211	5	39	123	25	67	119	0.147	0.853	0.277	0.723	0.0033
Zhang et al. [89]	Gene	2014	Asian	430	424	58	181	191	69	181	174	0.345	0.655	0.376	0.624	0.0630
Tao et al. [79]	Ann Rheum Dis	2007	Asian	220	203	39	95	49	39	108	51	0.472	0.527	0.47	0.53	0.2021
Enevold et al. [21]	Mol Biol Rep	2014	European	142	443	18	64	50	74	206	132	0.379	0.621	0.43	0.57	0.7629
Panda et al. [60]	Hum Immunol	2013	Asian	210	210	17	103	90	25	99	86	0.326	0.674	0.355	0.645	0.7634

Table 1 continued

(b) TLR9

Polymorphisms and study	Journals	Years	Ethnicity	Sample Size		Genotypes						Allele frequencies (%)				HWE
				Case	Control	Case			Control			Case		Control		
SNP rs187084						CC	CT	TT	CC	CT	TT	C	T	C	T	*P*
Ng et al. [55]	Rheumatology	2005	Asian	467	799	40	192	194	84	350	335	0.319	0.681	0.337	0.663	0.6289
Song and Song [76]	Chin J Lab Diagn	2009	Asian	92	88	17	38	37	22	41	25	0.391	0.609	0.483	0.517	0.5270
Ramachandran R [65]	Original article	2012	Asian	112	100	11	50	51	12	48	40	0.321	0.679	0.36	0.64	0.8285
Hur et al. [33]	Tissue Antigens	2005	Asian	350	330	44	124	80	50	162	102	0.427	0.572	0.417	0.583	0.2988
Rupasree et al. [69]	Lupus	2015	Asian	192	214	68	94	32	82	107	34	0.593	0.407	0.608	0.392	1.000
Zhong et al. [93]	Arthritis Res Ther	2011	Asian	545	487	78	256	211	67	228	192	0.378	0.622	0.372	0.628	1.000
SNP rs352140						TT	CT	CC	TT	CT	CC	T	C	T	C	
Zhang et al. [89]	Gene	2014	Asian	430	424	91	203	136	63	180	181	0.448	0.552	0.361	0.639	0.1139
Piotrowski et al. [62]	Rheumatol Int	2013	European	254	521	62	140	52	120	262	139	0.52	0.48	0.482	0.518	0.9301
Ng et al. [55]	Rheumatology	2005	Asian	467	799	38	199	184	86	328	335	0.327	0.673	0.334	0.666	0.6814
Zhou et al. [94]	Clin Exp Rheumatol	2010	Asian	315	338	49	141	125	53	176	109	0.379	0.621	0.417	0.583	0.2192
dos Santos et al. [18]	Lupus	2012	European	282	309	82	128	70	82	151	74	0.521	0.479	0.513	0.487	0.8193
dos Santos et al. [18]	Lupus	2012	African	88	106	14	40	34	13	47	42	0.386	0.614	0.358	0.642	1.000
Hur et al. [33]	Tissue Antigens	2005	Asian	350	330	61	168	108	52	145	101	0.43	0.57	0.418	0.582	1.000
Wen et al. [84]	Int J Clin Exp Med	2015	Asian	77	72	10	33	34	4	40	28	0.344	0.656	0.333	0.667	0.0608
Zhong et al. [93]	Arthritis Res Thera	2011	Asian	545	487	74	254	217	64	226	197	0.369	0.631	0.363	0.637	1.000
Lu et al. [45]	J Shanghai Jiaotong Univ	2007	Asian	26	12	3	13	10	2	1	9	0.365	0.635	0.208	0.792	0.0311
SNP rs5743836						CC	CT	TT	CC	CT	TT	C	T	C	T	
Zhang et al. [89]	Gene	2014	Asian	430	424	0	9	421	0	4	420	0.01	0.99	0.005	0.995	1.000
Enevold et al. [21]	Mol Biol Rep	2014	European	142	443	2	25	105	5	101	306	0.11	0.89	0.135	0.865	0.3980
Panda et al. [60]	Human Immunol	2013	Asian	210	210	2	38	170	6	32	172	0.1	0.9	0.105	0.895	0.0145
Ng et al. [55]	Rheumatology	2005	Asian	467	799	0	4	463	1	7	791	0.004	0.996	0.006	0.994	0.0224
Devaraju et al. [16]	Mol Immunol	2015	Asian	300	460	2	30	268	7	71	382	0.057	0.943	0.092	0.908	0.0927
Demirci FY [14]	J Rheumatol	2007	North American	398	540	10	106	282	15	141	348	0.158	0.842	0.17	0.83	0.8744
dos Santos et al. [18]	Lupus	2012	European	282	309	12	74	195	6	76	227	0.174	0.826	0.142	0.858	1.000

Table 1 continued

SNP rs5743836							CC	CT	TT	CC	CT	TT	C	T	C	T	
dos Santos et al. [18]	Lupus	2012	African		88	106	8	30	50	5	48	47	0.261	0.739	0.29	0.71	0.1442
SNP rs352139							GG	AG	AA	GG	AG	AA	G	A	G	A	
Zhang et al. [89]	Gene	2014	Asian		430	424	77	211	142	70	220	134	0.424	0.576	0.425	0.575	0.2331
Tao et al. [79]	Ann Rheum Dis	2007	Asian		220	203	56	115	49	37	105	61	0.516	0.484	0.441	0.559	0.5691
Ng et al. [55]	Rheumatology	2005	Asian		467	799	39	220	179	86	354	341	0.34	0.66	0.337	0.663	0.7486
Zhou et al. [94]	Clin Exp Rheumatol	2010	Asian		315	338	48	145	122	54	179	105	0.383	0.617	0.425	0.575	0.1478
Ramachandran et al. [65]	Original article	2012	Asian		112	100	5	99	8	12	76	12	0.487	0.513	0.5	0.5	0.000
Song and Song [77]	Med Lab Sci Clin	2011	Asian		92	88	22	49	21	12	46	30	0.505	0.495	0.398	0.602	0.5053
Hur et al. [33]	Tissue Antigens	2005	Asian		350	330	57	165	101	53	161	96	0.432	0.568	0.431	0.569	0.3540
Shahin et al. [74]	Immunol Investig	2016	African		104	108	8	86	10	32	62	14	0.49	0.51	0.583	0.417	0.0766

SNP single-nucleotide polymorphism; *HWE* Hardy–Weinberg equilibrium

3509 controls for rs187084, 2834 cases and 3398 controls for rs352140, 2317 cases and 3291 controls for rs5743836 and 2090 cases and 2390 controls for rs352139, respectively. The basic information of these included studies is shown in Table 1a, b.

Lastly, after excluding two groups of data in which the control populations deviated from HWE, 17 publications providing 9120 cases and 11,724 controls were pooled to evaluate the relationship between PTPN22 rs2476601 and SLE in the meta-analysis (Table 1a) [35]. Similarly, for TLR9 rs187084, rs352140, rs5743836 and rs352139, after exclusion of the studies of nonconformity with HWE, there were 2760 cases and 3298 controls, 2808 cases and 3386 controls, 1640 cases and 2282 controls and 1978 cases and 2290 controls (Table 1b), respectively.

Meta-analysis results

PTPN22 rs2476601 polymorphism and SLE risk

17 relevant studies including 9120 cases and 11,724 controls were analyzed in this meta-analysis after excluding two separate comparisons [35] of nonconformity with HWE. An outstanding association was found in the overall population, under the random effect model (T vs. C: OR = 1.511, 95% CI 1.338–1.706, $P = 2.931 \times 10^{-11}$, Table 2; Fig. 2a). Ethnicity-specific meta-analysis indicated significant association between rs2476601 and SLE in American and European populations (T vs. C: OR = 2.566, 95% CI 1.796–3.665, $P = 2.219 \times 10^{-7}$; OR = 1.399, 95% CI 1.261–1.552, $P = 2.153 \times 10^{-10}$, Table 2; Fig. 2d). We also identified an association between this polymorphism and SLE in Africans (T vs. C: OR = 4.14, 95% CI 1.753–9.775, $P = 0.001$, Table 2; Fig. 2d). However, no significant association was found between rs2476601 and SLE in Asians (T vs. C: OR = 1.323, 95% CI 0.550–3.181, $P = 0.532$, Table 2; Fig. 2d).

Accordingly, we used the dominant and recessive models to estimate the relationship between PTPN22 rs2476601 and SLE risk. We observed the association between this polymorphism and SLE in the dominant and recessive model of T allele in the overall population (TT+CT vs. CC: OR = 1.531, 95% CI 1.346–1.742, $P = 9.172 \times 10^{-11}$; TT vs. CT + CC: OR = 2.03, 95% CI 1.519–2.712, $P = 1.753 \times 10^{-6}$, Table 2; Fig. 2b, c). We also performed the stratification analysis by ethnicity of dominant and recessive models; similarly, there was a significant association between rs2476601 and SLE in the dominant model in the American, European and African populations. However, we found no association in the dominant and recessive models in Asians (Table 2; Supplementary Fig S1a, b).

Table 2 Meta-analysis of PTPN22 rs2476601 polymorphisms and SLE association

Population	No. of studies	T vs. C allele		TT+CT vs. CC (dominant model)		TT vs. CT+CC (recessive model)		Test of heterogeneity		Publication bias
		OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	*P*	I^2 (%)	Begg (*P*)
Overall	19	1.511 (1.338–1.706)	2.931×10^{-11}	1.531 (1.346–1.742)	9.17×10^{-11}	2.03 (1.519–2.712)	1.75×10^{-6}	0.020	44.2	0.050
American	4	2.566 (1.796–3.665)	2.219×10^{-7}	2.529 (1.740–3.675)	1.116×10^{-6}	9.672 (1.346–69.521)	0.024	0.908	0	1.000
European	11	1.399 (1.261–1.552)	2.153×10^{-10}	1.412 (1.267–1.575)	4.664×10^{-10}	1.953 (1.454–2.625)	8.996×10^{-6}	0.137	32.8	0.938
African	2	4.14 (1.753–9.775)	0.001	5.079 (2.053–12.569)	4.315×10^{-4}	NA	NA	0.508	0	1.000
Asian	2	1.323 (0.550–3.181)	0.532	1.255 (0.510–3.087)	0.621	1.557 (0.061–39.945)	0.789	0.953	0	1.000

OR odds ratio; *CI* confidence interval; *NA* not available (the number of TT genotype is 0, so the OR and *P* values could not be calculated)

TLR9 polymorphisms and SLE risk

The association between TLR9 rs187084 polymorphism and SLE was conducted in ten independent studies after the exclusion of one study [32] on the discrepancy of HWE. The test of heterogeneity in the overall population was not significant ($P = 0.732$, $I^2 = 0\%$), suggesting that the fixed effect model could be used. No association between the allele of TLR9 rs187084 and SLE was identified (C vs. T: OR = 0.929, 95% CI 0.860–1.003, $P = 0.061$, Table 3; Fig. 3a). We tested the dominant and recessive models of C allele in the overall population, but found no significant association in the two models (CC + CT vs. TT: OR = 0.913, 95% CI 0.817–1.019, $P = 0.104$; CC vs. CT+TT: OR = 0.899, 95% CI 0.776–1.042, $P = 0.158$, Table 3; Supplementary Fig S2a, b).

Similarly, we analyzed the cases and controls of TLR9 rs352140, rs5743836 and rs352139 to assess their association with SLE, after performing the HWE test and heterogeneity test. However, no association was found between rs352140, rs5743836, rs352139 and SLE susceptibility (Table 3; Fig. 3b–d). Furthermore, we analyzed the dominant and recessive models, respectively, which indicated that no statistically significant association was discovered in all of the dominant and recessive models (Table 3; Supplementary Figs S3a, b, S4a, b, S5a, b).

Allele frequency of the five SNPs and comparison to the 1000 Genomes Phase 3 population

We displayed the allele frequencies in different ethnicities in our meta-analysis and 1000 genomes of the five SNPs in Table 4. In view of the sample size and population, the allele frequencies of PTPN22 rs2476601, TLR9 rs187084 and TLR9 rs352139 in this meta-analysis were consistent with the allele frequencies in the 1000 Genome Project AMR (Admixed American), EUR (European ancestry), AFR (African ancestry), EAS (East Asian ancestry), respectively, however, there was distinction between the allele frequencies of TLR9 rs352140, TLR9 rs5743836 and 1000 Genomes Project (Table 4).

Publication bias and sensitivity analysis

Begg's funnel plot was used to evaluate the publication bias. There was no obvious evidence of symmetry from the shapes of the funnel plots (Fig. 4a–e) and no evidence of publication bias of all study subjects ($P > 0.05$). Accordingly, we performed sensitivity analysis to assess the influence of individual study on the pooled OR. The analysis showed that no individual study significantly affected the pooled OR; therefore, no study was deleted (Fig. 5a–e).

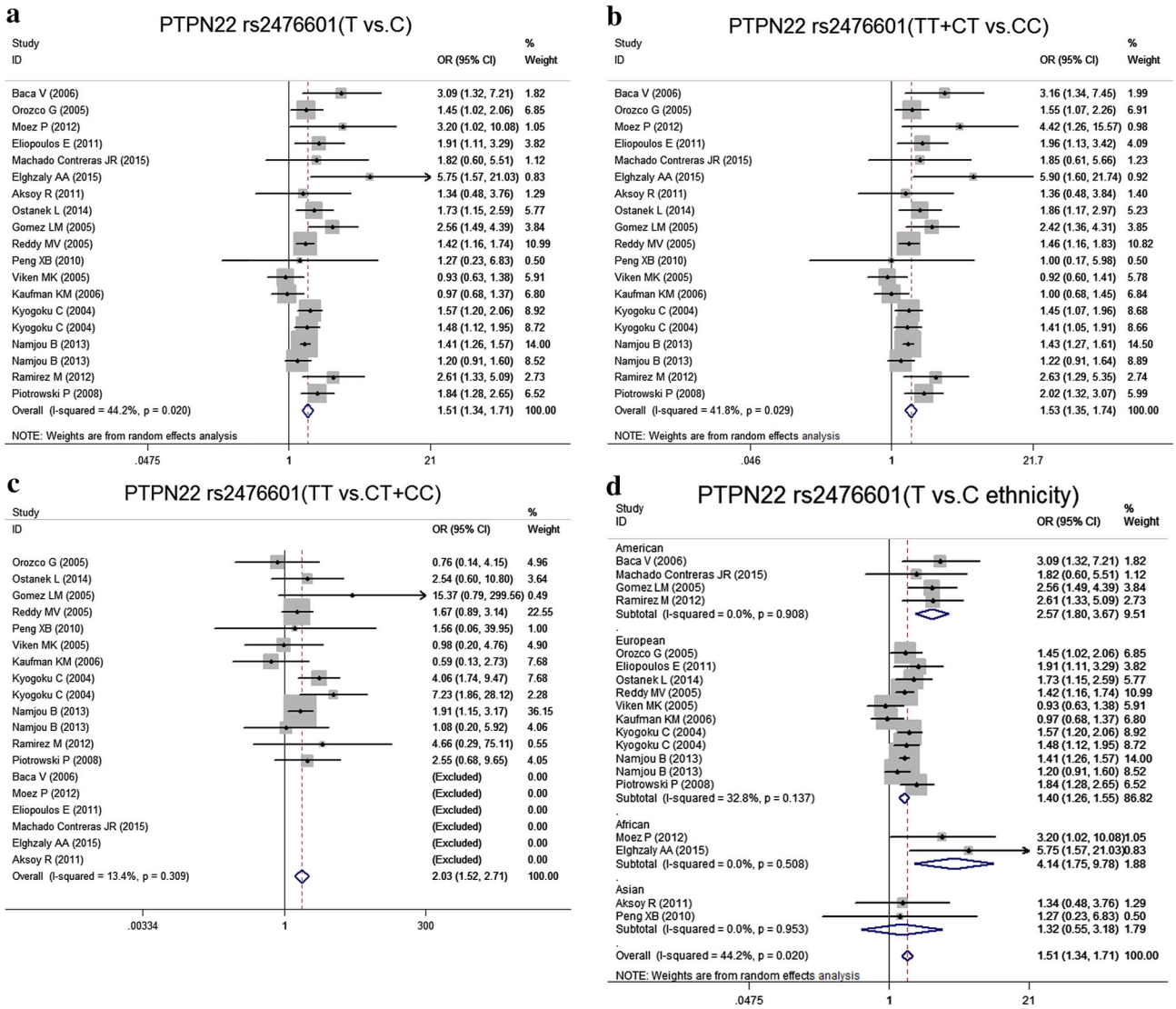

Fig. 2 Forest plot for the meta-analysis of the association between PTPN22 rs2476601 and SLE **a** rs2476601 and SLE (T vs. C), **b** rs2476601 and SLE (TT+CT vs. CC), **c** rs2476601 and SLE (TT vs. CT+CC), **d** rs2476601 and SLE after stratification by ethnicity (T vs. C)

Discussion

In this study, we conducted a meta-analysis of the association between PTPN22 rs2476601 and TLR9 polymorphisms with SLE susceptibility. We validated that the T allele of PTPN22 rs2476601 apparently increased the risk of SLE, but its effect was diverse in different ethnics. Although we found no significant association between TLR9 polymorphisms and SLE, we summed up and verified previous studies.

SNP rs2476601 is a missense mutation in the PTPN22 gene on chromosome 1p13.3-13.1, which was identified to be associated with multiple autoimmune diseases [10]. In 2004, PTPN22 rs2476601 was first indicated to be associated with type 1 diabetes [7] and rheumatoid arthritis [5]; afterward, studies showed the relevance between PTPN22 and other diseases, such as SLE, myasthenia gravis and Crohn's disease [17, 38, 81]. Recently, GWAS have discovered significant association between PTPN22 rs2476601 and SLE in Europeans [49]. To check this association in different ethnicities, we carried out a comprehensive ethnicity-specific meta-analysis involving 9120 cases and 11,724 controls with 19 separate comparisons, and found that the association between PTPN22 rs2476601 and SLE achieved genome-wide significance in the overall population and reached significant correlation in the American and European, and a certain relevance in the African, but not in the Asian population. Zheng et al. [92] previously carried out a meta-analysis with 14 case–control studies and testified a significant association between PTPN22 rs2476601 and SLE, acquiring a more significant result than those in our meta-analysis. However, after

Table 3 Meta-analysis of TLR9 gene polymorphisms and SLE association

Polymorphism	Comparison	Test of association			Test of heterogeneity			Publication bias
		OR	95% CI	*P*	Model	*P*	I^2 (%)	Begg (*P*)
TLR9 rs187084	C versus T	0.929	0.860–1.003	0.061	F	0.732	0	0.074
	CC + CT versus TT	0.913	0.817–1.019	0.104	F	0.903	0	0.049
	CC versus CT+TT	0.899	0.776–1.042	0.158	F	0.823	0	0.371
TLR9 rs352140	T versus C	1.069	0.961–1.188	0.22	R	0.06	46.6	0.754
	TT+CT versus CC	1.076	0.91–1.273	0.391	R	0.026	54	0.754
	TT versus CT+CC	1.1	0.958–1.264	0.175	F	0.341	11.3	0.917
TLR9 rs5743836	C versus T	0.915	0.712–1.176	0.486	R	0.047	55.5	1.000
	CC + CT versus TT	0.867	0.661–1.137	0.303	R	0.067	51.5	1.000
	CC versus CT+TT	1.201	0.745–1.935	0.453	F	0.344	10.9	0.806
TLR9 rs352139	G versus A	1.018	0.878–1.181	0.81	R	0.02	60	1.000
	GG+GA versus AA	1.06	0.868–1.296	0.567	R	0.067	49	0.548
	GG versus GA+AA	0.957	0.673–1.360	0.805	R	0.001	73.5	1.000

TLR9 toll-like receptor 9; *OR* odds ratio; *CI* confidence interval; *F* fixed effect model; *R* random effect model; I^2 the effective value of heterogeneity

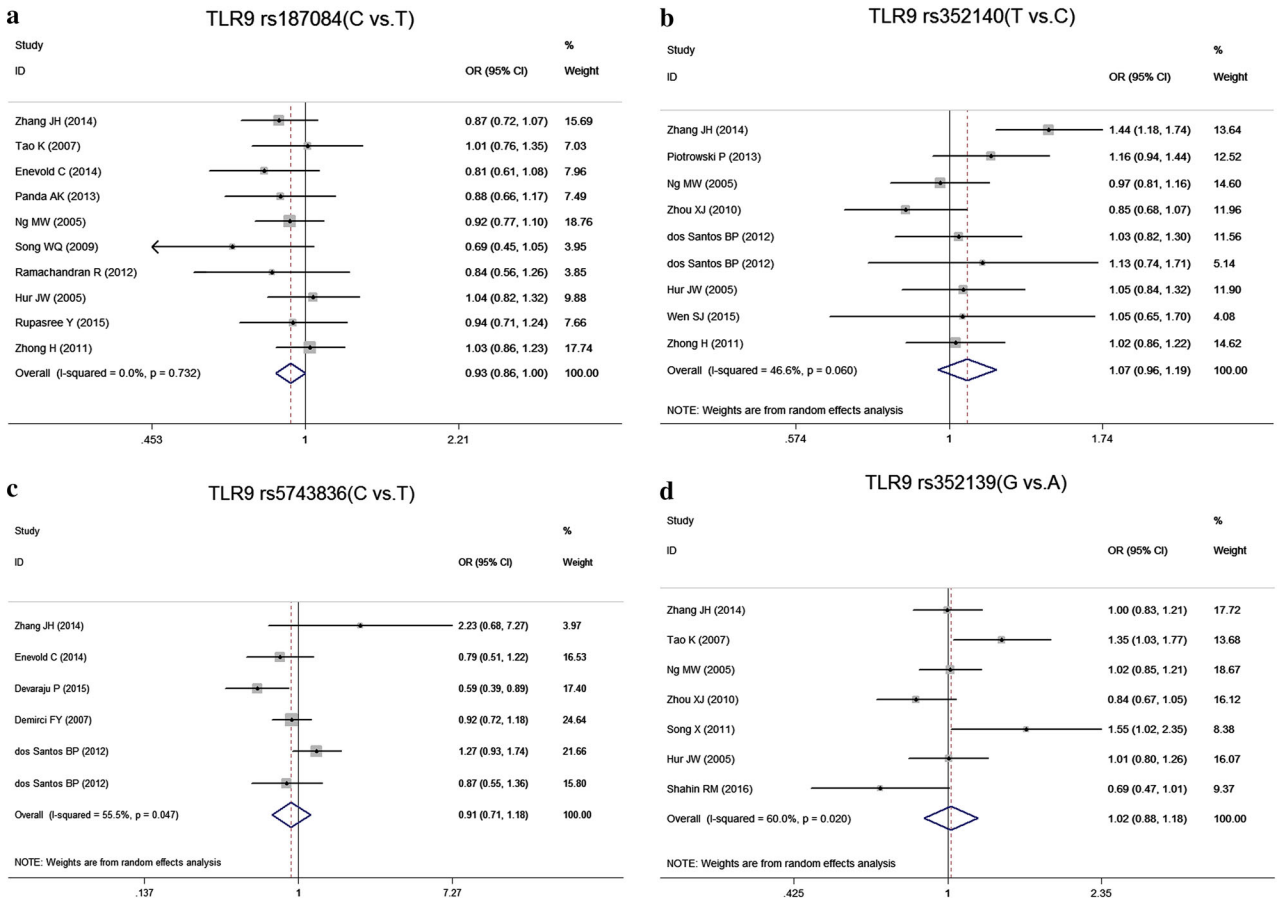

Fig. 3 Forest plot for the meta-analysis of the association between TLR9 polymorphisms and SLE **a** TLR9 rs187084 and SLE (C vs. T), **b** TLR9 rs352140 and SLE (T vs. C), **c** TLR9 rs5743836 and SLE (C vs.T) and **d** TLR9 rs352139 and SLE (G vs. A)

Table 4 The allele frequency comparison between the meta-analysis and 1000 Genomes Project

Polymorphism	Populations	Meta-analysis (alleles frequencies)				1000 genomes (alleles frequencies)	
		Case		Control			
PTPN22 rs2476601		T	C	T	C	T	C
	American	0.055	0.945	0.026	0.974	0.04 (AMR)	0.96 (AMR)
	European	0.104	0.896	0.085	0.915	0.09 (EUR)	0.91 (EUR)
	African	0.074	0.926	0.011	0.989	0 (AFR)	1.00 (AFR)
	Asian	0.037	0.963	0.025	0.975	0 (EAS)	1.00 (EAS)
	All	0.099	0.901	0.076	0.924	0.03	0.97
TLR9 rs187084		C	T	C	T	C	T
	Asian	0.385	0.615	0.395	0.605	0.40 (EAS)	0.60 (EAS)
	European	0.379	0.621	0.43	0.57	0.43 (EUR)	0.57 (EUR)
	All	0.384	0.616	0.4	0.6	0.38	0.62
TLR9 rs352140		T	C	T	C	T	C
	Asian	0.387	0.613	0.367	0.633	0.39 (EAS)	0.61 (EAS)
	European	0.521	0.479	0.493	0.507	0.55 (EUR)	0.45 (EUR)
	African	0.386	0.614	0.358	0.642	0.29 (AFR)	0.71 (AFR)
	All	0.413	0.587	0.399	0.601	0.42	0.58
TLR9 rs5743836		C	T	C	T	C	T
	Asian	0.029	0.971	0.05	0.95		
	European	0.154	0.846	0.138	0.862	0.13 (EUR)	0.87 (EUR)
	North America	0.158	0.842	0.17	0.83	0.11 (AMR)	0.89 (AMR)
	African	0.261	0.739	0.29	0.71	0.42 (AFR)	0.58 (AFR)
	All	0.105	0.895	0.117	0.883	0.17	0.83
TLR9 rs352139		G	A	G	A	G	A
	Asian	0.413	0.587	0.394	0.606	0.40 (EAS)	0.60 (EAS)
	African	0.49	0.51	0.583	0.417	0.61 (AFR)	0.39 (AFR)
	All	0.418	0.582	0.403	0.597	0.51	0.49

PTPN22 protein tyrosine phosphatase nonreceptor22, *TLR9* toll-like receptor 9, *AMR* Admixed American, *EUR* European ancestry, *AFR* African ancestry, *EAS* East Asian ancestry, *ALL* all individuals from Phase 3 of the 1000 Genomes Project

careful reading, we found that Zheng et al. included a study of rheumatoid arthritis [20] and a study with data deviated from HWE [35]. Papers of this kind were not supposed to pass the inclusion criteria of meta-analysis; we therefore excluded these two studies and included an additional seven newly published studies after Zheng's meta-analysis. Meta-analysis by Lee et al. [43], Shi et al. [75] and Lea et al. [40] included only 6, 4 and 11 studies, mainly in European, respectively. Our meta-analysis was more comprehensive and more diverse in population.

There were only three studies [19, 35, 48] for the African population included in this meta-analysis (one paper was excluded because of HWE deviation [35]), including only 210 effective cases and 261 controls. However, the effect of the T allele in the African population is in the same direction as that in the overall population. It was easy for us to see that rs2476601 T allele frequency was low in all the populations, and it was monomorphic in the African and Asian populations in the 1000 Genomes Phase 3 (Table 4). We could notice that the T allele was not monomorphic in the two African studies, and the T allele frequency of SLE case from Moez et al. [48] was much higher than that from Elghzaly et al. [19] (0.263 vs 0.029) (Table 1a). Because only 40 SLE cases were included in the study from Moez et al., the frequency was not reliable. Additionally, Sanchez et al. [71] have reported that no association was found between PTPN22 rs2476601 and SLE in African-Americans. These phenomenon and discrepancy needed further investigation on the basis of large sample size.

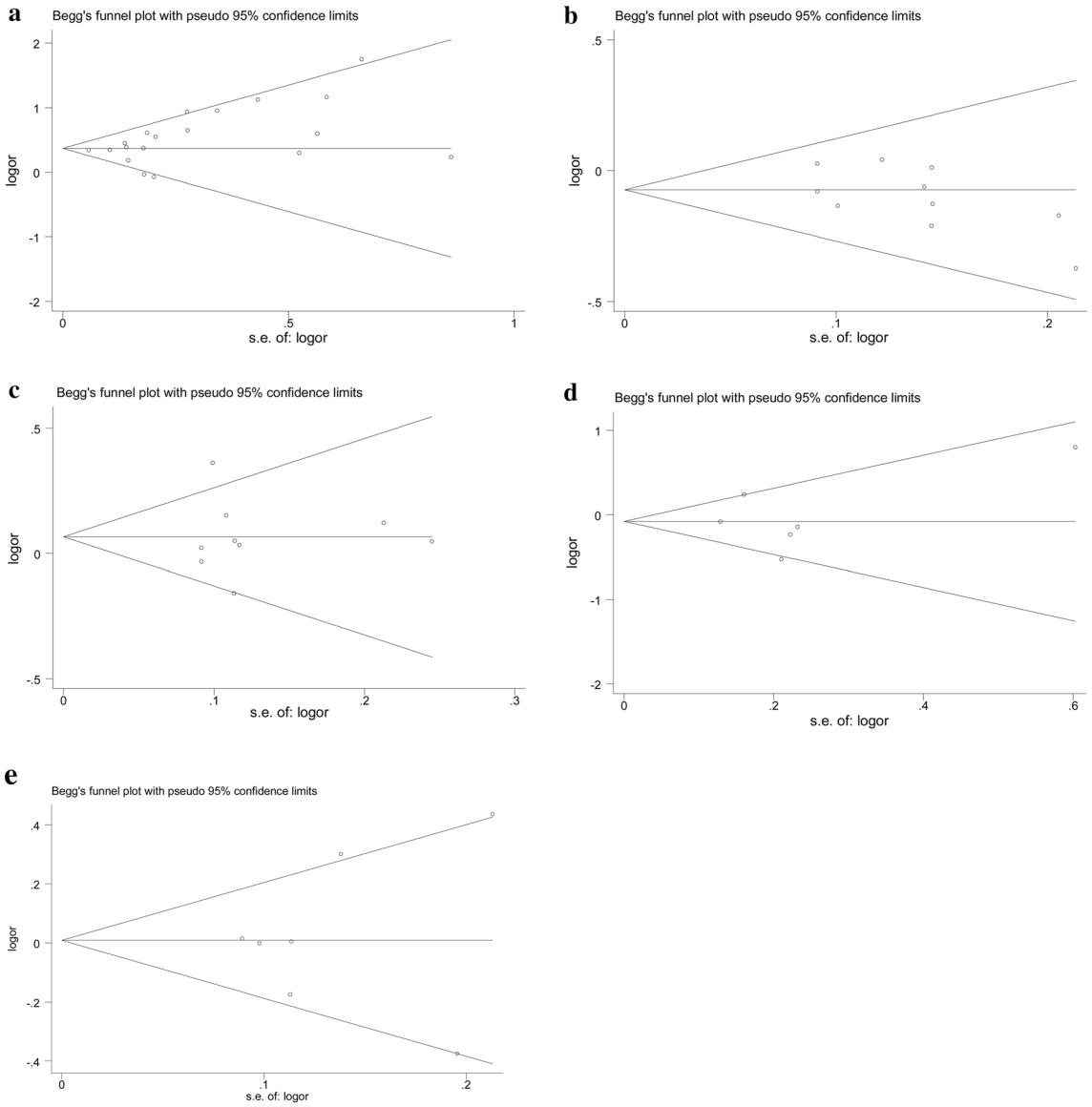

Fig. 4 Begg's funnel plot of publication bias in the meta-analysis of the association of PTPN22 and TLR9 polymorphisms with SLE risk under allele genetic model. **a** PTPN22 rs2476601 and SLE (T vs. C), **b** TLR9 rs187084 and SLE (C vs.T), **c** TLR9 rs352140 and SLE (T vs. C), **d** TLR9 rs5743836 and SLE (C vs. T) and **e** TLR9 rs352139 and SLE (G vs. A)

Previous studies have showed that genotype TT was not detected in the Chinese population [8, 91], similarly, no rs2476601 T allele and structural variant R620W of the PTPN22 protein were found in the Asian population [36, 41]. As shown in the 1000 Genomes Phase 3, the T allele was also not existent in the East Asian population (Table 4). Therefore, theoretically speaking, no association should be detected between rs2476601 and SLE in Asians. Although our result is consistent with the theory, more studies need to evaluate their association.

TLR9 could modulate autoimmunity by inducing inflammatory cells and by the production of cytokines or antibodies [79]. The expression and mRNA level of TLR9 on B cell were up-regulated in SLE patients, and SLE activity index was significantly associated with TLR9 expression [51]. Some studies indicated that TLR9 expression was obviously higher in SLE cases than in healthy controls. Wang et al. [83] performed a meta-analysis of TLR9 polymorphisms and SLE risk, and 21 studies from 19 articles with 10,273 subjects were included in that meta-analysis. The results indicated that TLR9 rs187084 could increase the risk of SLE (T vs. C: OR = 1.15, 95% CI 1.02–1.30, $P = 0.020$) in Asians with marginal significance and no evidence was found for the association between TLR9 rs352140, rs5743836, rs352139 and SLE risk. Similarly, Lee et al. [42] carried out a meta-analysis

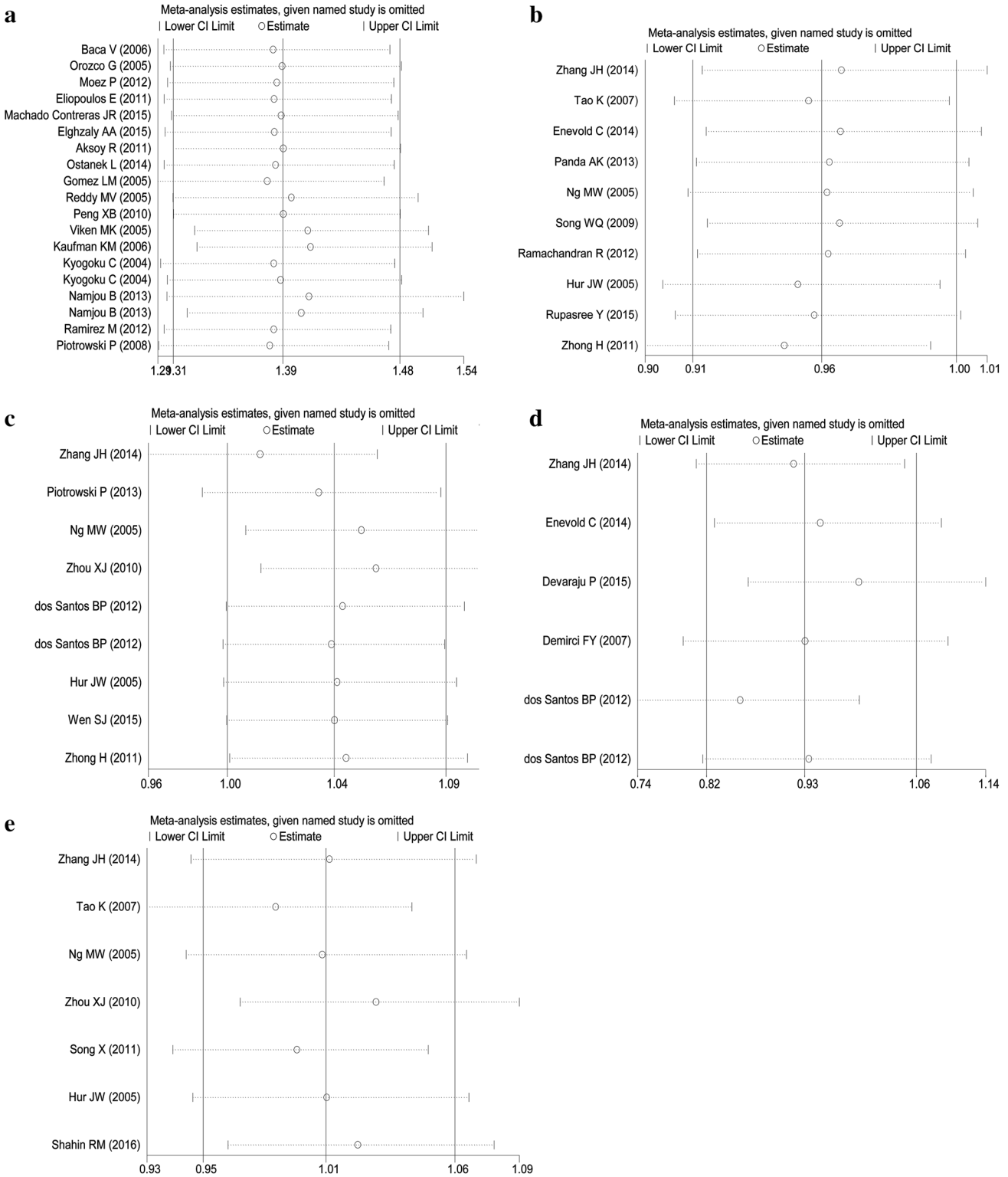

Fig. 5 Sensitivity analysis to assess the stability of the meta-analysis. **a** PTPN22 rs2476601 in SLE, **b** TLR9 rs187084 in SLE, **c** TLR9 rs352140 in SLE, **d** TLR9 rs5743836 in SLE and **e** TLR9 rs352139 in SLE

and the results showed that only TLR9 rs187084 was associated with SLE risk (OR = 0.869, 95% CI 0.762–0.992, $P = 0.038$). Although they verified the relativity of SLE and TLR9 rs187084, under the circumstances of $P = 0.02$ and $P = 0.038$, we considered the relevance was marginal rather than significant. However,

Li et al. and Yang et al. indicated that none of these three polymorphisms (rs187084, rs352139, rs352140) showed any significant association with SLE risk in Asian populations [44, 87].

To precisely explore the association between TLR9 polymorphisms (rs187084, rs352140, rs5743836, rs352139) and SLE, we analyzed the data that were consistent with HWE and used the random effect model to deal with the heterogeneity. Ultimately, 18 articles with 10,690 subjects were analyzed comprehensively. Although TLR9 has been evidenced to be responsible for the maturation of dendritic cells (DCs) and the production of pro-inflammatory cytokines in knockout mice [29], we have no sufficient information to identify the significant relationship between TLR9 and SLE in the study population. Then, we compared our study with the meta-analysis from Wang et al. [83] and found that they included two groups of repeated data [76, 77], which could lead to imprecise results. On comparing our data with that of Lee et al. [42], we found that a study with data deviating from HWE ($P < 0.05$) was included in Lee et al., and we included one additional newly published study [93] which led to the diversity of results. Simultaneously, we realized that marginal significance was reached under the allele model and dominant model for TLR9 rs187084 if we included the data of nonconformity with HWE in the population (C vs. T: OR = 0.877, 95% CI 0.783–0.982, $P = 0.022$;CC + CT vs. TT: OR = 0.875, 95% CI 0.787–0.974, $P = 0.014$, Supplementary Fig S6a and Fig S6b) and in Asians (C vs. T: OR = 0.881 95% CI 0.780–0.996, $P = 0.043$; CC + CT vs. TT: OR = 0.883, 95% CI 0.791–0.986, $P = 0.027$, Supplementary Fig S7a, b), which indicated that the association between rs187084 and SLE was suggestive.

Although there were new discoveries revealed by this study, several potential limitations existed. Firstly, we need a large number of data to validate the relationship between PTPN22 and SLE for further study in the African and other populations. For TLR9, most studies included in this meta-analysis were carried out in Asian populations, so we had insufficient data to conduct subgroup meta-analysis. In other words, available data could not confirm the significant association between TLR9 and SLE. Secondly, we had to point out that significant between-study heterogeneity that was detected. We used the random effect model to deal with the heterogeneity, but this might introduce an insensible statistical illusion because fixed effect model and random effect model address different research questions [50]. Despite these deficiencies, we had searched for information and analyzed data as comprehensively as possible.

In conclusion, our meta-analysis indicated that the missense SNP rs2476601 in PTPN22 was significantly associated with SLE susceptibility in the overall population, and the association had reached genome-wide significance. However, the relationship between Africans and Asians needs further investigation. TLR9 polymorphisms rs187084, rs352140, rs5743836 and rs352139 may not be associated with SLE.

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Compliance with ethical standards

Conflict of interest The authors have no competing interests that might be perceived to influence the results and/or discussion reported in this paper. This study re-analyzed the previous published data; therefore, this study did not involve original human participants.

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