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B-cell lymphocyte kinase polymorphisms rs13277113, rs2736340, and rs4840568 and risk of autoimmune diseases

A meta-analysis

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Abstract

Background: B-cell lymphocyte kinase (BLK) is an inhibitor of B cells that has an important influence on several autoimmune diseases, but there is a lack of comprehensive analysis of its association with autoimmune diseases. Hence, it is meaningful to conduct a comprehensive analysis.

Methods: A systematic literature search was performed on the PubMed, ScienceDirect, and Web of Science databases up to June 30, 2016. The data were extracted and quality-assessed before conducting the meta-analysis. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were assessed with the STATA version 12.0 software. Subgroup and sensitivity analysis were conducted to explore potential sources of heterogeneity.

Results: Altogether, 33 studies with 68,874 cases and 90,684 controls, 24 studies with 31,095 cases and 39,077 controls for rs13277113, 21 studies with 26,388 cases and 40,635 controls for rs2736340, and 4 studies with 11,391 cases and 10,972 controls for rs4840568 were included in this meta-analysis. The results revealed that the BLK rs13277113 and rs2736340 polymorphisms increased the risk of autoimmune diseases in the total analysis (A vs G: OR = 1.33, 95% CI = 1.27–1.39, $P < .01$; T vs C: OR = 1.34, 95% CI = 1.27–1.41, $P < .01$), and rs4840568 was positively associated with systemic lupus erythematosus (SLE) (A vs G: OR = 1.32, 95% CI = 1.22–1.43, $P = .01$).

Conclusion: This meta-analysis shows that the BLK (rs13277113, rs2736340, rs4840568) polymorphisms may be a risk factor for developing autoimmune diseases, especially for Asian populations and SLE.

Abbreviations: 95% CIs = 95% confidence intervals, BCR = pre-B cell receptor, BLK = B-cell lymphocyte kinase, dcSSc = diffuse cutaneous SSc, DM = dermatomyositis, GCA = giant cell arteritis, HC = hospital control, HWE = Hardy–Weinberg equilibrium, lcSSc = limited cutaneous SSc, MMN = multifocal motor neuropathy, NOS = Newcastle–Ottawa scale, ORs = odds ratios, PC = population control, PM = polymyositis, PSS = primary Sjögren syndrome, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SSc = systemic sclerosis.

Keywords: autoimmune disease, B-cell lymphocyte kinase, BLK, meta-analysis, polymorphism

1. Introduction

Autoimmune diseases are a clinical manifestation of a broken immune tolerance and intermittent inflammation, which leads to significant mortality and morbidity.[1] Nearly 100 autoimmune diseases have been discovered, and autoimmune diseases can occur at any age. Epidemiological studies have shown that autoimmune diseases affect 3% to 5% of the general population.[2] The prevalence is increased in woman, with the ratio of affected females to males ranging from 10:1 to 1:1.[3–5] Generally, a broken immune tolerance is the key cause for autoimmune diseases. Immune tolerance is the ability to prevent the immune system from targeting self-molecules, cells, or tissues.[6] When immune tolerance has been destroyed and self-reactive lymphocytes contribute to inflammation, a classical or pathological autoimmune disease develops. In this process, B cells, T cells, antigen-presenting cells, and effector cells all regulate the manifestation of autoimmune diseases.[7–9]

BLK is located on the short arm of chromosome 8 (8p23.1) and is one of the tyrosine kinases that transduce signals downstream of the B-cell receptor.[10] A genome-wide association study (GWAS) confirmed that a single-nucleotide polymorphism (SNP) in the 5′ upstream region of the *BLK* gene was associated with

SLE.[11] Multiple studies found that BLK was also associated with other autoimmune diseases.[12] Studies have shown that BLK is mainly expressed in B-cells and plays an important role in the proliferation and differentiation of B cells.[13] Wu et al[14] found that BLK risk genotypes in mice and humans were correlated with an increased subset of B1a or B1-like cells. Moreover, the expression of IgG anti-dsDNA antibodies in healthy individuals with a BLK risk genotype was significantly increased.[14] Therefore, through regulating immunological components, the BLK risk genotypes might contribute to the development of autoimmune disease.

The A allele of rs13277113, T allele of rs2736340, and A allele of rs4840568 are located in the promoter region of BLK and inhibit the expression of BLK mRNA. Studies have shown that the BLK rs13277113 "A" allele was associated with an increased risk of a number of autoimmune diseases and other disorders, including SLE,[15] systemic sclerosis (SSc),[16] and rheumatoid arthritis (RA).[17] Many randomized controlled trials (RCTs) have explored the relationship between BLK rs13277113 and other autoimmune diseases, such as polymyositis (PM),[18] dermatomyositis (DM),[18] multifocal motor neuropathy (MMN),[19] and giant cell arteritis (GCA).[20] Several studies have also shown that BLK rs2736340 was associated with SLE,[21] and Kawasaki disease,[22] and is possibly involved in RA,[23] PM,[24] DM,[24] GCA,[20] primary Sjögren syndrome (PSS),[25] primary antiphospholipid syndrome (PAS),[26] and SSc.[27] Until now, studies found that BLK rs4840568 was merely related to SLE.[28] In 2011, Fan et al[15] performed a meta-analysis of 7 case–control studies for rs13277113 and found that the A allele of rs13277113 was a risk factor for SLE. Recently, another meta-analysis of 21 case–control studies for rs2736340 by Zhou et al,[29] published in 2016, found that the rs2736340 polymorphism is associated with several autoimmune diseases. Small sample size and the low statistical power of previous studies contributed to our interest in conducting a comprehensive and precise analysis of the association between BLK polymorphisms and autoimmune diseases. This meta-analysis was conducted based on the meta-analysis of observational studies in epidemiology (MOOSE) guidelines.

2. Methods

Ethical approval was not unnecessary, as this study was a meta-analysis that collects and analyzes data from the existing literatures.

2.1. Eligibility criteria

The eligible studies met the following criteria were considered to include the study type is a case–control study or cohort study, investigated the association of the BLK (rs13277113, rs2736340, rs4840568) polymorphisms with autoimmune diseases susceptibility, included accurate genotype distribution data for each group or sufficient data to calculate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs), and there were no limiting conditions regarding language. We excluded the abstracts, overlapped studies, and the study lack of key data.

2.2. Search strategy

The association between the BLK (rs13277113, rs2736340, rs4840568) polymorphisms and autoimmune diseases susceptibilities was explored by a comprehensive search. PubMed, ScienceDirect, and Web of Science were searched up to June 30,

2016, to identify the studies that used the following keywords: "BLK," "B-cell lymphocyte kinase," "autoimmune diseases," "polymorphism," "mutation," "variant." All of the included studies were published.

2.3. Data extraction

The following information was extracted independently by 2 authors (Zeng and Weng) with discussion: surname first author, publication year, country, the racial descent of the subjects, the source of the controls, the number of cases and controls with each genotype, Hardy–Weinberg equilibrium (HWE) among the controls, genotyping method, and type of diseases. The x^2 test was used to estimate whether the genotype frequencies in the control groups accorded with HWE.

2.4. Quality assessment

Two independent authors (Zeng and Xu) evaluated the included case control studies using the Newcastle–Ottawa scale (NOS) for quality assessment. The scores on this scale range from 0 to 9 points. Studies with scores of 6 or higher were considered high quality; otherwise, they were identified as low quality.[30]

2.5. Statistical analysis

ORs with 95% CIs were calculated to assess the association between the BLK (rs13277113, rs2736340, rs4840568) polymorphisms and autoimmune diseases susceptibility. For the (rs13277113, rs2736340, rs4840568) polymorphisms, pooled ORs were used for the allele contrast (A vs G or T vs C), homozygote contrast (AA vs GG or TT vs CC), heterozygote contrast (AG vs GG or TC vs CC), the dominant model (AA+AG vs GG or TT+TC vs CC), and the recessive model (AA vs AG + GG or TT vs TC+CC). Subgroup analysis was based on the HWE status of control, ethnicity, the source of the controls, and types of diseases. The values of $P < .1$ and $I^2 > 50\%$ were used to indicate the absence or existence of obvious heterogeneity; hence, a random-effect model was adopted. Otherwise, the fixed-effects model was used. Publication bias was analyzed with funnel plots. All of the statistical analyses were conducted using STATA version 12.0 (Stata Corporation, College Station, TX).

3. Results

3.1. Characteristics of included studies

The initial literature search identified 84 relevant articles from the PubMed, ScienceDirect, and Web of Science databases. On the basis of title screening and duplications, 39 publications were excluded because of an irrelevant theme or were a duplicate study. Twelve publications were discarded because of irrelevant location or inaccurate data. This resulted in 33 significant articles for inclusion in our meta-analysis (Fig. 1).[11,12,16,17,19,20,23–28,31–51] The specific characteristics and quality scores are provided in Table 1. All of the studies were case–control studies. Of these 33 articles, 4 articles reported on different countries,[11,17,27,34] 2 articles reported on different ethnicities,[31,42] 4 articles reported on different types of systemic scleroses or idiopathic inflammatory myopathies,[24,27,35,44] and 13 articles reported on different sites of BLK polymorphisms[11,20,23–25,27,28,33,36,38,41–43] and were treated as independent studies. Therefore, we divide the 33 articles into 66 specific case–control studies of BLK (68,874 cases and 90,684 controls).
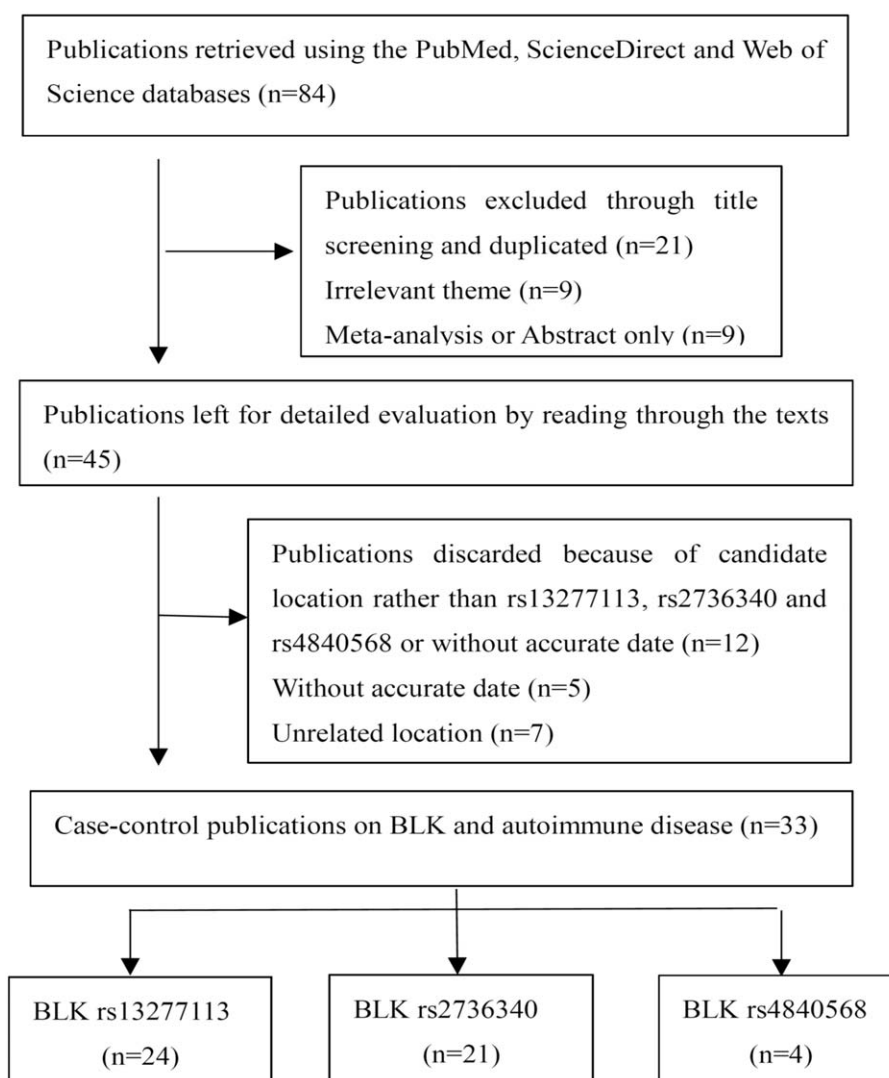
Figure 1. Flow diagram of the study selection process.

3.2. Meta-analysis

The association between the rs13277113 polymorphism and autoimmune diseases risk is illustrated in Table 2. When these types of autoimmune diseases were viewed as a whole, we found that the combined analysis revealed a mildly increased risk of autoimmune diseases in 5 genetic models for BLK rs13277113 (A vs G: OR = 1.33, 95% CI 1.27–1.39, $P < .01$; AG vs GG: OR = 1.31, 95% CI 1.26–1.37, $P = .6$; AA vs GG: OR = 1.76, 95% CI 1.60–1.93, $P < .01$; AA+AG vs GG: OR = 1.41, 95% CI 1.33–1.51, $P < .01$; AA vs AG+GG: OR = 1.48, 95% CI 1.41–1.56, $P = .16$, Table 2, Fig. 2). The subgroup analysis by ethnicity indicated that the A allele was associated with an increased risk in Caucasians, Asians, and Africans (Table 2). It deserves to be mentioned that positive correlations with autoimmune diseases were observed in SLE (A vs G: OR = 1.39, 95% CI 1.34–1.44, $P = .2$). An association with dcSSc and lcSSc was apparent (A vs G: OR = 1.27, 95% CI 1.04–1.54, $P = .16$; A vs G: OR = 1.25, 95% CI 1.05–1.49, $P = .15$), as well as RA (A vs G: OR = 1.22, 95% CI 1.01–1.48, $P = .02$). The results of the subgroup analysis reveal that there are associations of the

BLK rs13277113 polymorphism with PPS, PM, and DM but not with GCA (Table 3). However, due to small sample sizes, some of results were not statistically significant, such as MMN (Table S1, http://links.lww.com/MD/B856). Therefore, the heterogeneity may have been derived from ethnicity, the source of the control, and the types of diseases and so on.

For rs2736340, the pooled analysis merging all of the studies suggested a slight association with autoimmune diseases in 5 genetic models (T vs C: OR = 1.34, 95% CI = 1.27–1.41, $P < .01$; TC vs CC: OR = 1.27, 95% CI 1.21–1.33, $P = .18$; TT vs CC: OR = 1.73, 95% CI 1.54–1.94, $P < .01$; TT+TC vs CC: OR = 1.39, 95% CI 1.30–1.49, $P < .01$; TT vs TC+CC: OR = 1.49, 95% CI 1.39–1.59, $P = .02$, Table 2, Fig. 3). The subgroup analysis by ethnicity revealed that the T allele was a risk allele in Caucasians and Asians, but not in Africans. Positive correlations with autoimmune diseases were observed in SLE (OR = 1.38, 95% CI = 1.32–1.44, $P = .26$), RA (OR = 1.24, 95% CI = 1.12–1.37, $P < .01$), Kaw (OR = 1.55, 95% CI = 1.36–1.76, $P = .8$), and lcSSc (OR = 1.33, 95% CI = 1.16–1.52, $P = .8$) (Table 3). As before, the results of GCA and dcSSc were not

Table 1

Characteristics of case–control studies on BLK (rs13277113, rs2736340, rs4840568) polymorphisms and autoimmunity disease risk included in the meta-analysis.

First author	Year	Country	Racial	Design	Case	Control	HWE*	Genetic variant	Types	NOS
Hom et al[11]	2008	Sweden/USA	Caucasian	PC	793/1311	857/3340	0.99	rs13277113, rs2736340	SLE	7
Han et al[33]	2009	China	Asian	HC	4199	8255	0.98	rs13277113, rs2736340	SLE	7
Yin et al[26]	2009	Italy	Caucasian	PC	130	395	0.69	rs2736340	PAS	7
Ito et al[31]	2009	Japan	Asian/Caucasian	HC	327/1311	322/3336	0.99/0.83	rs13277113	SLE	6
Gregersen et al[12]	2009	North American	Caucasian	PC	2645	7047	0.99	rs2736340	RA	8
Suarez-Gestal et al[32,45]	2009	Spain	Caucasian	PC	1412/3268	1512/810	0.98/0.95	rs13277113	SLE/RA	7
Yang et al[34]	2009	China/Thailand	Asian	HC	910/278	1440/383	0.99/0.38	rs13277113	SLE	6
Ito et al[35,46]	2010	Japan	Asian	PC	603/309	492/769	0.89/0.99	rs13277113	RA/SSc	7
Freudenberg et al[47]	2010	Korea	Asian	PC	801	757	0.95	rs2736340	RA	8
Torres et al[20]	2010	Spain	Caucasian	HC	218	486	0.21	rs13277113, rs2736340	GCA	6
Gourh et al[27]	2010	North American/Spain	Caucasian	PC	986/471	692/702	0.38/0.16	rs13277113, rs2736340	SSc	7
Yang et al[40]	2010	China	Asian	PC	320	1500	0.99	rs2736340	SLE	8
Coustet et al[16]	2011	France	Caucasian	PC	313/628	978	0.78	rs13277113	dcSSc/lcSSc	7
Deshmukh et al[23]	2011	Colombia	Caucasian	PC	353	368	0.99	rs13277113, rs2736340	RA	7
Orozco et al[48]	2011	UK	Caucasian	PC	3962	9275	0.99	rs2736340	RA	8
Vlam et al[19]	2011	Dutch	Caucasian	PC	91	1078	0.76	rs13277113	MMN	7
Jarvinen et al[28]	2012	Sweden	Caucasian	HC	275	356	0.97	rs13277113, rs2736340, rs4840568	SLE	6
Lee et al[50]	2012	China	Asian	PC	833	1657	0.97	rs2736340	Kaw	7
Zhou et al[43]	2012	China	Asian	PC	804	722	0.95	rs13277113, rs2736340	SLE	7
Chen et al[38]	2012	China	Asian	HC	532	576	<0.05	rs13277113, rs4840568	SLE	5
Genin et al[17]	2013	France/Japan/Spain	Caucasian	PC	806/634/461	1203/322/373	0.56/0.62/0.24	rs13277113	RA	7
Sun et al[25]	2013	China	Asian	HC	533	564	0.47	rs13277113, rs2736340	PSS	6
Kadota et al[37]	2013	Japan	Asian	PC	75	190	0.23	rs13277113	SLE	7
Namjou et al[41]	2014	USA	Caucasian	PC	3926	3490	0.99	rs13277113, rs2736340, rs4840568	SLE	8
Guthridge et al[42]	2014	USA	Caucasian/African/Asian	PC	3980/1406/1272	3546/1734/1270	0.97/0.97/0.98	rs13277113, rs2736340, rs4840568	SLE	8
Shu et al[36]	2014	China	Asian	PC	130	163	0.94	rs13277113, rs2736340	SSc	7
Sugiura et al[44]	2014	Japan	Asian	PC	283/194	656	0.98	rs13277113	PM/DM	7
Elghzaly et al[51]	2015	Egypt	African	HC	170	241	<0.05	rs2736340	SLE	5
Jin et al[49]	2015	China	Asian	PC	184	203	0.06	rs2736340	Kaw	6
Dang et al[39]	2015	China	Asian	HC	382	660	0.97	rs2736340	SLE	6
Chen et al[24]	2015	China	Asian	HC	310/535	968	0.96	rs13277113, rs2736340	PM/DM	7

dcSSc = diffuse cutaneous SSc, DM = dermatomyositis, GCA = giant cell arteritis, HWE = Hardy–Weinberg equilibrium, Kaw = Kawasaki, lcSSc = limited cutaneous SSc, MMN = multifocal motor neuropathy, NOS = Newcastle–Ottawa scale, PAS = primary antiphospholipid syndrome, PM = polymyositis, PSS = primary Sjögren syndrome, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SSc = systemic sclerosis.

* P value for Hardy–Weinberg equilibrium in control.

statistically significant, larger sample sizes are needed (Table S1, http://links.lww.com/MD/B856).

As for rs4840568, we identified 6 studies, including 11,391 cases and 10,972 controls. Results revealed that rs4840568 polymorphism increased the risk of SLE in the total analysis (A vs G: OR = 1.32, 95% CI 1.22–1.43, $P < .01$; AG vs GG: OR = 1.28, 95% CI 1.20–1.38, $P = .32$; AA vs GG: OR = 1.74, 95% CI 1.47–2.06, $P = .04$; AA+AG vs GG: OR = 1.40, 95% CI 1.25–1.57, $P = .02$; AA vs AG+GG: OR = 1.48, 95% CI 1.36–1.60, $P = .43$, Table 2, Fig. 4).

Table 2

Summary ORs and 95% CI of BLK (rs13277113, rs2736340, rs4840568) polymorphisms and autoimmune disease risk by ethnicity and source of controls.

Rs13277113	N	A vs G OR	95% CI	P	I²	AG vs GG OR	95% CI	P	I²	AA vs GG OR	95% CI	P	I²	AA+AG vs GG OR	95% CI	P	I²	AA vs AG+GG OR	95% CI	P	I²
Total	33	1.33	1.27–1.39	<.01	55	1.31	1.26–1.37	.6	0	1.76	1.60–1.93	<.01	40	1.41	1.33–1.51	<.01	49	1.48	1.41–1.56	.16	20
Caucasian	15	1.26	1.18–1.34	<.01	64	1.29	1.22–1.36	.2	22	1.59	1.39–1.83	.23	17	1.32	1.23–1.41	.01	52	1.43	1.27–1.61	.03	45
Asian	17	1.41	1.35–1.48	.3	13	1.40	1.26–1.55	.9	0	1.98	1.80–2.19	.45	0.7	1.71	1.55–1.89	.6	0	1.50	1.43–1.58	.59	0
African	1	1.38	1.20–1.58	—	—	1.38	1.17–1.62	—	—	1.89	1.18–3.02	—	—	1.42	1.21–1.65	—	—	1.73	1.09–2.77	—	—
PC	22	1.32	1.26–1.38	.02	—	1.3	1.24–1.36	.85	0	1.72	1.57–1.89	.23	17	1.37	1.29–1.45	.15	24	1.51	1.42–1.61	.51	0
HC	11	1.34	1.23–1.46	<.01	68	1.36	1.21–1.52	.2	25	1.81	1.48–2.22	<.01	60	1.52	1.29–1.80	<.01	65	1.45	1.31–1.60	.03	49
rs2736340	N	T vs C				TC vs CC				TT vs CC				TT+TC vs CC				TT vs TC+CC			
Total	27	1.34	1.27–1.41	<.01	70	1.27	1.21–1.33	.18	20	1.73	1.54–1.94	<.01	61	1.39	1.30–1.49	<.01	59	1.49	1.39–1.59	.02	41
Caucasian	13	1.28	1.20–1.37	<.01	75	1.26	1.19–1.34	.04	45	1.64	1.41–1.91	<.01	69	1.32	1.23–1.43	<.01	68	1.47	1.30–1.66	.007	56
Asian	12	1.43	1.36–1.52	.37	8	1.33	1.15–1.54	.74	0	1.94	1.68–2.24	.49	0	1.67	1.45–1.92	.55	0	1.53	1.43–1.63	.54	0
African	2	1.25	0.97–1.62	.13	56	1.37	1.18–1.60	.83	0	1.34	0.64–2.82	.06	70	1.38	1.19–1.60	.37	0	1.24	0.6–2.56	.07	70
PC	19	1.36	1.28–1.44	<.01	75	1.28	1.22–1.35	.11	30	1.81	1.60–2.06	<.01	63	1.42	1.31–1.53	<.01	67	1.51	1.40–1.59	.03	42
HC	8	1.28	1.13–1.45	.04	53	1.14	0.97–1.34	.58	0	1.43	1.10–1.87	.13	38	1.25	1.06–1.48	.32	15	1.40	1.19–1.65	.09	43
rs4840568	N	A vs G				AG vs GG				AA vs GG				AA+AG vs GG				AA vs AG+GG			
Total	6	1.33	1.22–1.43	<.01	66	1.28	1.20–1.38	.32	15	1.74	1.47–2.06	.04	56	1.40	1.25–1.57	.02	63	1.48	1.36–1.60	.43	0
Caucasian	3	1.29	1.23–1.36	.67	0	1.30	1.21–1.38	.80	0	1.67	1.48–1.88	.76	0	1.35	1.27–1.44	.74	0	1.49	1.33–1.67	.83	0
Asian	2	1.52	1.36–1.69	.85	0	1.64	1.23–2.21	.44	0	2.41	1.81–3.21	.74	0	2.08	1.57–2.76	.58	0	1.56	1.38–1.80	.5	0
African	1	1.15	1.03–1.27	—	—	1.15	0.99–1.36	—	—	1.31	1.04–1.64	—	—	1.18	1.02–1.36	—	—	1.22	0.98–1.51	—	—
PC	4	1.30	1.19–1.42	<.01	75	1.27	1.20–1.35	.40	0	1.65	1.39–1.97	.02	62	1.34	1.21–1.50	.04	64	1.46	1.32–1.63	.20	35
HC	2	1.47	1.26–1.72	.77	0	1.56	1.18–2.07	.32	0	2.36	1.58–3.51	.56	0	1.80	1.18–2.74	.15	52	1.51	1.21–1.89	.60	0

95% CI = 95% confidence intervals, HC = hospital control, HWE = Hardy–Weinberg equilibrium, OR = odds ratios, PC = population control.
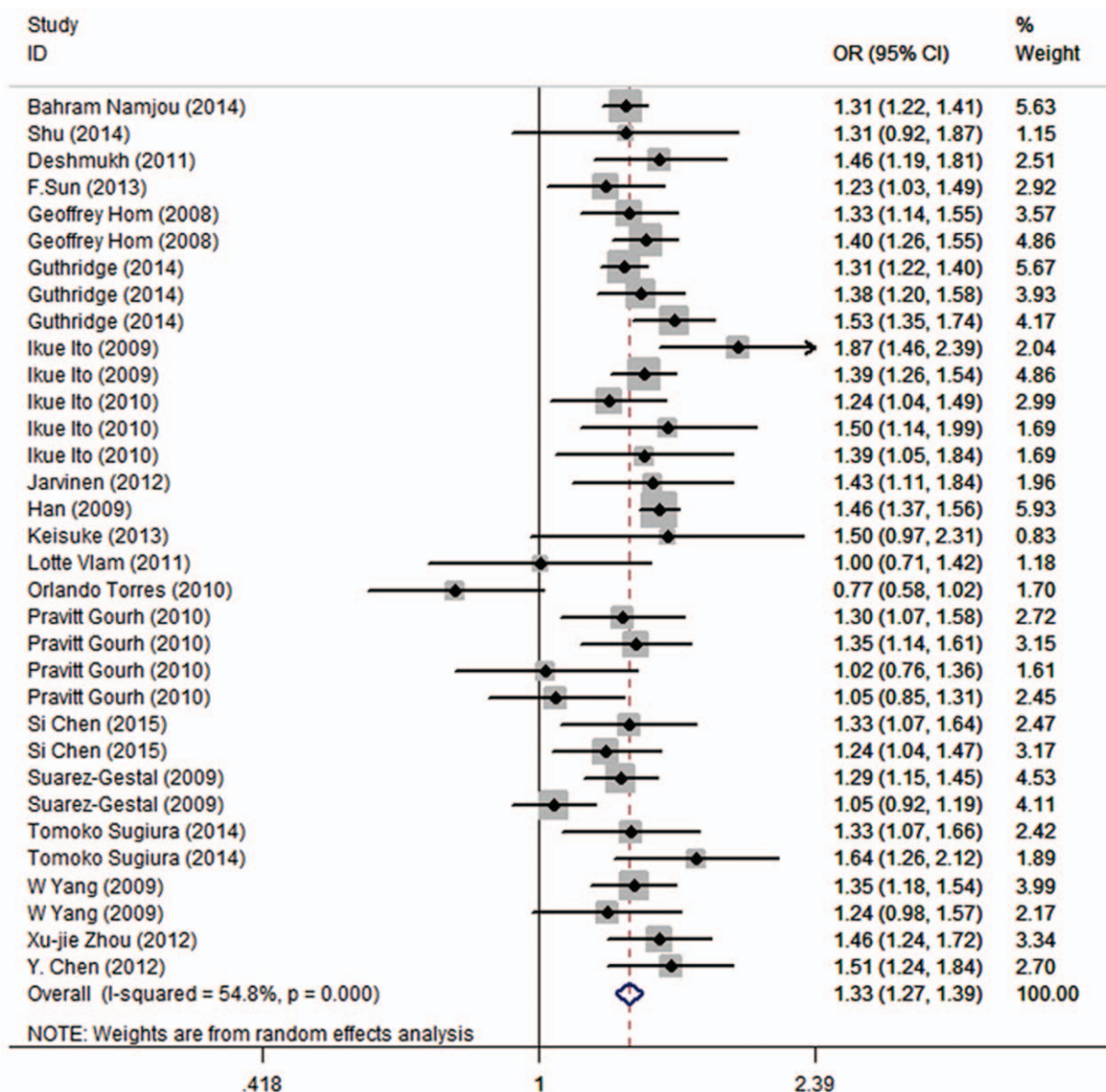
Figure 2. Calculated ORs and 95% CIs for the association between BLK rs13277113 polymorphism and autoimmune diseases risk in the A versus G model. 95% CIs = 95% confidence intervals, ORs = odds ratios.

3.3. Sensitivity analysis and Publication bias

In the sensitivity analysis, the pooled ORs were not substantially altered when any single study was excluded, which confirms that the results of this meta-analysis are statistically stable. For rs13277113 and rs4840568, the funnel plots displayed symmetrical shapes (Fig. 5). This result was supported by statistical evidence from Egger test ($P = .269$; $P = .338$), but for rs2736340, Egger test showed a publication bias ($P = .013$). The "trim and fill" method was used to mirror the asymmetric studies by imputing hypothetical negative unpublished studies. The pooled results with merged hypothetical studies still show an association with autoimmune diseases (OR = 1.32, 95% CI 1.25–1.39, $P < .01$), and the funnel plots using the "trim and fill" method displayed symmetrical shapes (Fig. 6).

4. Discussion

Genetic factors have become increasingly important in the progression of autoimmune diseases. In the pathogenesis and clinical manifestations of autoimmune disease, immune complexes in the bloodstream consisting of auto-antibody and antigen complexes lead to inflammation of the kidneys, brain, and skin.[11] BLK is a member of the Src family of tyrosine kinases, which is involved in signal transduction downstream of the B-cell receptor (BCR).[18] Previous studies have discovered the pathogenesis of mutant BLK in which the BCR triggers the phosphorylation of tyrosine residues on immunoreceptor via BLK and other Src family tyrosine kinases, inducing the phosphorylation and activation of tyrosine-protein kinase Syk. Syk is the key factor in initiating the pre-BCR signaling cascade, which leads to B cell activation and B cell–T cell interaction.[23] In

Table 3

Meta-analysis for the association between BLK (rs13277113, rs2736340, rs4840568) polymorphism and autoimmune diseases by types of diseases.

	N	OR	95% CI	P	I²	OR	95% CI	P	I²	OR	95% CI	P	I²	OR	95% CI	P	I²	OR	95% CI	P	I²
rs13277113	38		A vs G				AG vs GG				AA vs GG				AA+AG vs GG				AA vs AG+GG		
SLE	17	1.39	1.34–1.43	.2	24	1.36	1.30–1.42	.93	0	1.91	1.78–2.06	.53	0	1.50	1.40–1.60	.03	44	1.55	1.48–1.62	.90	0
dcSSc	3	1.27	1.04–1.54	.16	45	1.17	0.95–1.43	.94	0	1.48	0.84–2.60	.09	58	1.24	1.02–1.51	.7	0	1.42	0.90–2.25	.07	62
lcSSc	3	1.25	1.05–1.49	.15	48	1.25	1.05–1.48	.39	0	1.45	1.05–2.00	.3	15	1.28	1.04–1.57	.26	25	1.40	1.10–1.78	.36	3
RA	3	1.22	1.01–1.48	.02	74	1.17	0.95–1.44	.2	38	1.49	1.01–2.20	.06	64	1.28	0.96–1.71	.04	68	1.32	1.05–1.66	.16	20
PSS	1	1.23	1.03–1.49	—	—	0.96	0.61–1.50	—	—	1.31	0.84–2.03	—	—	1.14	0.74–1.75	—	—	1.36	1.07–1.72	—	—
MMN	1	1.0	0.71–1.42	—	—	0.94	0.38–2.33	—	—	0.96	0.4–3.23	—	—	0.95	0.4–2.26	—	—	1.02	0.66–1.56	—	—
GCA	1	0.77	0.58–1.02	—	—	0.90	0.64–1.27	—	—	0.37	0.15–0.91	—	—	0.81	0.58–1.13	—	—	0.39	0.16–0.94	—	—
PM	2	1.33	1.43–1.55	.97	0	1.32	0.90–1.94	.94	0	1.76	1.20–2.58	.94	0	1.55	1.01–2.24	.98	0	1.40	1.15–1.69	.94	0
DM	2	1.40	1.06–1.84	.08	68	1.37	0.93–2.0	.5	0	1.93	1.12–3.33	.17	48	1.64	1.10–2.44	.28	14	1.47	1.06–2.03	.09	65
rs2736340	27		T vs C				TC vs CC				TT vs CC				TT + TC vs CC				TT vs TC+CC		
SLE	12	1.38	1.32–1.44	.26	19	1.33	1.26–1.41	.99	0	1.81	1.65–2.00	.55	0	1.41	1.34–1.48	.54	0	1.58	1.48–1.70	.79	0
RA	4	1.24	1.12–1.37	<.01	79	1.19	1.08–1.31	.10	52	1.54	1.23–1.92	<.01	74	1.27	1.12–1.45	.08	75	1.33	1.17–1.52	.11	50
PSS	1	1.23	1.02–1.49	—	—	0.83	0.52–1.35	—	—	1.18	0.74–1.89	—	—	1.02	0.65–1.62	—	—	1.37	1.00–1.74	—	—
PAS	1	2.06	1.53–2.78	—	—	1.73	1.12–2.67	—	—	4.89	2.49–9.61	—	—	2.11	1.41–3.16	—	—	3.82	2.01–7.26	—	—
Kaw	2	1.55	1.36–1.76	.8	0	1.49	1.05–2.13	.74	0	2.32	1.64–3.27	.82	0	1.96	1.40–2.76	.82	0	1.64	1.41–1.92	.83	0
GCA	1	0.90	0.69–1.18	—	—	0.98	0.70–1.36	—	—	0.68	0.33–1.43	—	—	0.93	0.67–1.30	—	—	0.69	0.33–1.42	—	—
dcSSc	2	1.18	0.99–1.39	.43	0	1.18	0.95–1.47	.99	0	1.33	0.82–2.14	.27	18	1.21	0.98–1.49	.71	0	1.24	0.77–2.10	.26	20
lcSSc	2	1.33	1.16–1.52	.8	0	1.35	1.03–1.78	.99	0	1.66	1.21–2.29	.64	0	1.40	1.18–1.66	.92	0	1.47	1.01–2.01	.62	0
PM	1	1.41	1.13–1.77	—	—	1.75	0.89–3.42	—	—	2.33	1.21–4.49	—	—	2.09	1.09–4.00	—	—	1.43	1.10–1.86	—	—
DM	1	1.23	1.03–1.47	—	—	1.14	0.71–1.80	—	—	1.43	0.91–2.24	—	—	1.31	0.84–2.03	—	—	1.28	1.03–1.60	—	—
rs4840568	6		A vs G				AG vs GG				AA vs GG				AA+AG vs GG				AA vs AG+GG		
SLE	6	1.33	1.22–1.43	<.01	66	1.28	1.20–1.38	.32	15	1.74	1.47–2.06	.04	56	1.40	1.25–1.57	.02	63	1.48	1.36–1.60	.43	0

95% CI = 95% confidence intervals, dcSSc = diffuse cutaneous SSc, DM = dermatomyositis, GCA = giant cell arteritis, HWE = Hardy–Weinberg equilibrium, Kaw = Kawasaki, lcSSc = limited cutaneous SSc, MMN = multifocal motor neuropathy, NOS = Newcastle–Ottawa scale, OR = odds ratios, PAS = primary antiphospholipid syndrome, PM = polymyositis, PSS = primary Sjögren syndrome, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SSc = systemic sclerosis.
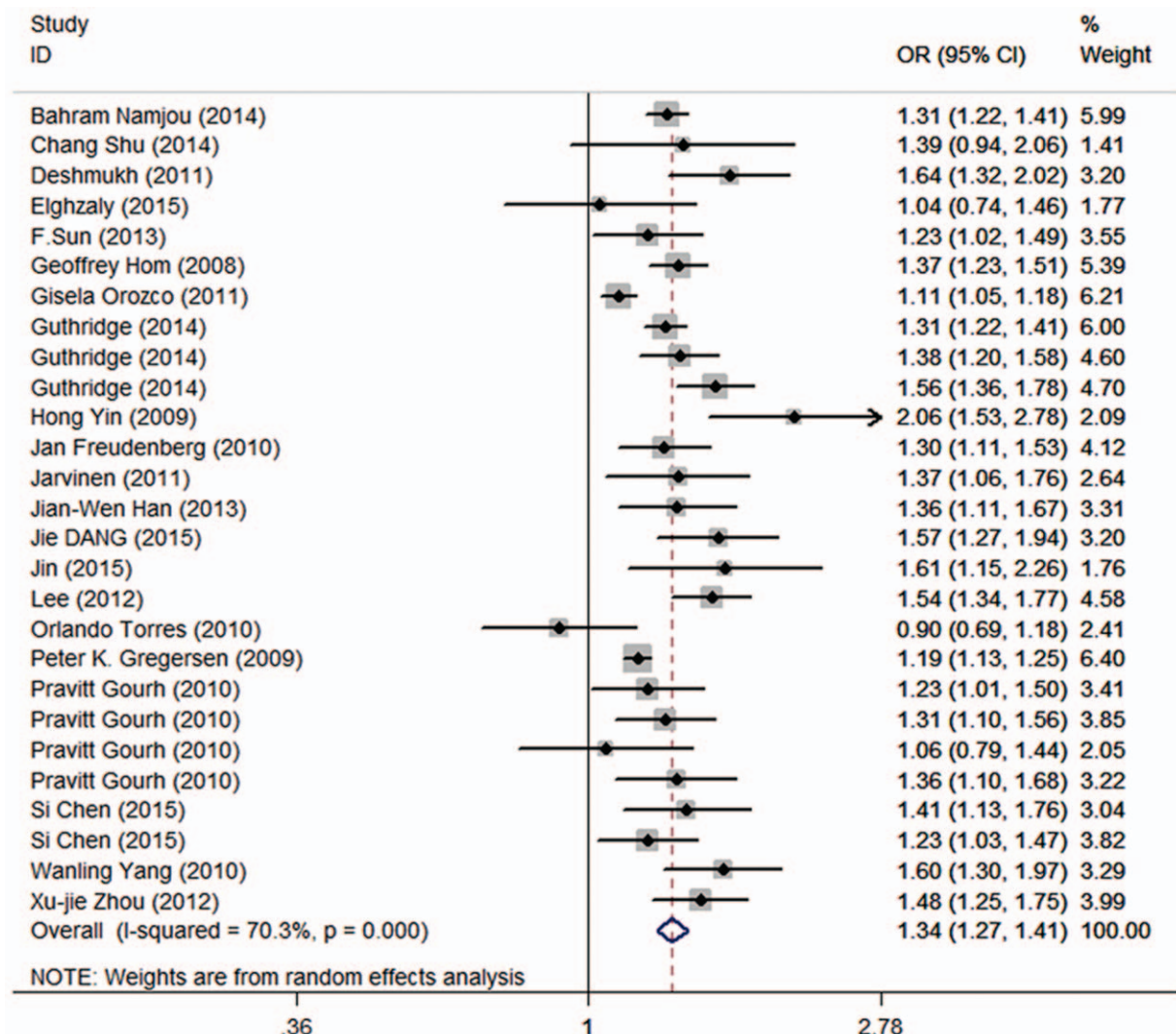

Figure 3. Calculated ORs and 95% CIs for the association between BLK rs2736340 polymorphism and autoimmune diseases risk in the T versus C model. 95% CIs = 95% confidence intervals, ORs = odds ratios.
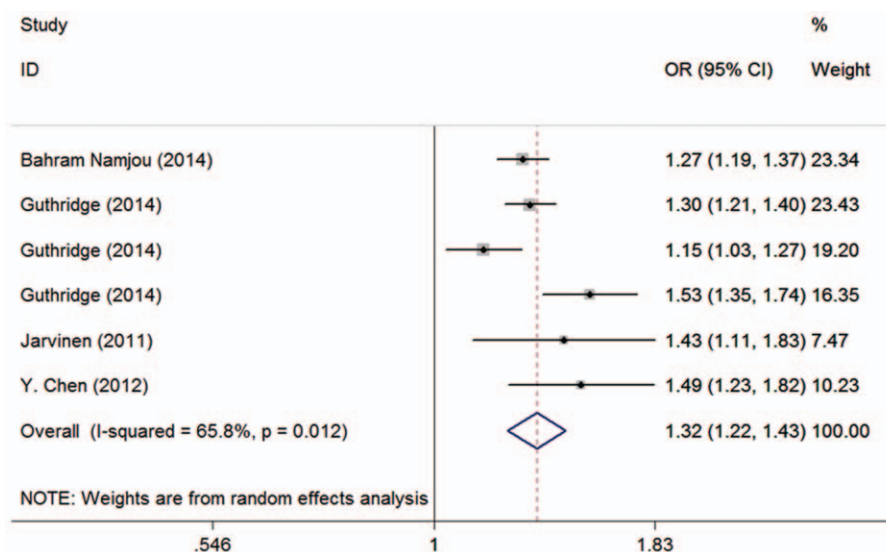
Figure 4. Calculated ORs and 95% CIs for the association between BLK rs4840568 polymorphism and autoimmune diseases risk in the A versus G model. 95% CIs=95% confidence intervals, ORs=odds ratios.

other words, the BLK risk genotypes lower the baseline for activation of B lymphocytes and assist in the communication between B lymphocytes and T lymphocytes. Thus, BLK has been deemed a meaningful candidate gene for autoimmune diseases.[27,52]

The findings from this meta-analysis, based on 68,874 cases and 90,684 controls of autoimmune diseases patients, found that the BLK polymorphisms rs13277113, rs2736340, and rs4840568 are significantly associated with susceptibility to autoimmune disease. The results were in accordance with previous studies and might provide a new biomarker in the etiology of autoimmune diseases. We also conducted subgroup analyses to further explore the potential effects of the patients' ethnicities, the source of the controls, and the types of illness with the association of BLK polymorphisms and autoimmune diseases. The results revealed that BLK polymorphisms were closely associated with the risk of autoimmune diseases in all ethnicities; however, the correlation was stronger in Asian group

than in other racial groups. Our studies also found that the BLK polymorphisms rs13277113, rs2736340, and rs4840568 are unevenly distributed in terms of ethnicity. In Caucasians and Africans, the A and T allele of BLK are the minor alleles, but they are the major alleles in Asians. Even so, the results indicated that the A allele of BLK rs13277113 and rs4840568 and the T allele of BLK rs2736340 were a risk factor for autoimmune diseases. The reason for this finding may be genetic disparities between the ethnic groups.[15] Due to the process of natural selection, different groups might have some differences in the functional variants.[51] In addition, the A allele of rs13277113 is a risk factor for SLE, diffuse cutaneous SSc (dcSSc), PSS, PM, and DM but is a protective factor for limited cutaneous SSc (lcSSc), RA, SSc, MMN, and GCA. As Zhou et al[29] demonstrated, the T allele of rs2736340 is a risk factor in SLE, RA, Kawasaki disease, and lcSSc but was ambiguous with other diseases. Although autoimmune diseases have similar pathogenic mechanisms, there are some factors that cause differences in these diseases. This may
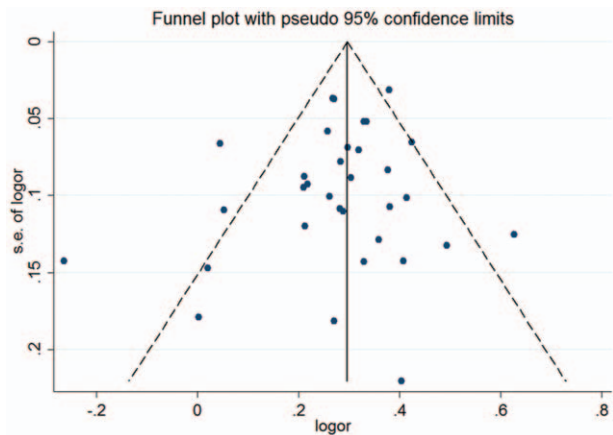

Figure 5. Funnel plot analysis to detect publication bias for A versus G model of BLK rs13277113 polymorphism circles represent the weight of the studies. ORs=odds ratios.
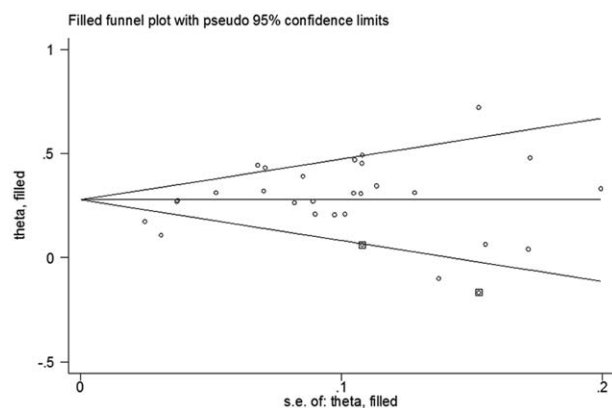

Figure 6. Funnel plot analysis with "trim and fill" method to expected publication bias for A versus G model of BLK rs2736340 polymorphism circles represent the weight of the studies. ORs=odds ratios.

be attributed to the fact that the majority of autoimmune diseases have multiple genetic factors that play a role.[2] The number of studies included in this meta-analysis is an important aspect of these results. Our research found that BLK can act as an influencing factor on autoimmune diseases. Mutations of these SNPs can be used as a risk factor for assessing autoimmune diseases. Furthermore, on the basis of the pathogenicity of BLK polymorphisms, our research provides a meaningful therapeutic strategy for autoimmune diseases.

Recently, a similar meta-analysis by Zhou et al has been published online (https://www.ncbi.nlm.nih.gov/pubmed/27105348). We analyzed their well-designed study and found some advantages of our meta-analysis. In their study, they conducted a meta-analysis focusing on 21 studies and concluded that the BLK rs2736340 polymorphism is associated with several autoimmune diseases. We conducted a meta-analysis using 27 studies and obtained similar conclusions. However, the methodology assessment and sources of heterogeneity of the included studies in our analysis were more detailed than in the previous study (Tables 1 and 3). In addition, we also included 33 studies of BLK rs13277113 and 6 studies of BLK rs4840568 to systematically explore the relationship between BLK and autoimmune diseases. Therefore, a larger sample size and more SNP types are included in the present meta-analysis.

Nevertheless, some limitations should be acknowledged. First, the possible effects of gene–gene and gene–environmental interactions on the risks of autoimmune diseases were not estimated due to the limited information available in the original papers. Second, publication bias may exist because we only included published articles that were identified through online database searching. Third, high heterogeneity was observed in all 5 genetic models of 2 types of gene polymorphism. Subgroup analysis indicated that the heterogeneity might have been derived from the design of the controls, the ethnicities, and the types of diseases.

5. Conclusion

This meta-analysis indicated that the BLK (rs13277113, rs2736340, rs4840568) polymorphisms were associated with an increased risk of autoimmune diseases. In a word, because of the limitation in the included studies, more studies are essential for a more accurate conclusion.

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