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Original Article

Association between tumor necrosis factor-α gene polymorphisms and diffuse large B-cell lymphoma in Chinese Han population: evidence from two center case-control study and a meta-analysis

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Abstract: Objectives: The tumor necrosis factor-α (TNF-α) gene, which plays crucial roles in tumorigenesis, is reported to be an independent marker for cancer. This study aims to examine the association between the TNF-α G308A polymorphism and DLBCL risk based on the two center case-control studies and meta-analysis. Methods: In the current study, we performed a two centers case-control study to investigate the effect of the TNF-α G308A polymorphism on DLBCL risk in Chinese Han population. A meta-analysis including 10 published datasets along with current dataset, including 111 comparisons containing 34,041 cases and 42,730 controls were enrolled, was next performed to further confirm the association after literature search was conducted and relevant studies were identified from PubMed, Embase, and Web of Science. Results: The TNF-α -308A allele was associated with a significantly increased DLBCL risk in the two independent patient case-control studies and additionally for pooled analysis from the two sets ($P<0.05$ for both). The result of meta-analysis further demonstrated that the A allele of -308A was significantly correlated with DLBCL risk under the allelic model (OR=1.35, 95% CI=1.27-1.44) without heterogeneity by fixed-effects model analysis (Q=17.30, $P=0.139$). Moreover, sensitivity analysis supported the robustness of this meta-analysis. Conclusion: This study suggested that -308A polymorphism may be associated with the susceptibility of DLBCL in a Chinese population. The further meta-analysis provides additional evidence supporting the above result that the risk allele of the -308A polymorphism may increase DLBCL risk.

Keywords: *TNF-α,* polymorphism, diffuse large B-cell lymphoma

Introduction

Diffuse large B cell lymphomas (DLBCL) is the most common lymphoid malignancy worldwide, accounting for 30-35% of non-Hodgkin lymphoma cases [1, 2] comparison of incidence patterns by disease subtype may provide critical clues for future etiologic investigations. We therefore conducted a comprehensive assessment of 114,548 lymphoid neoplasms diagnosed during 1992-2001 in 12 Surveillance, Epidemiology, and End Results (SEER and definitely the most common of the aggressive types of lymphoma, the annual incidence of which is reported to be 15-20 cases/100,000, and 37% of B cell tumors worldwide [3].

DLBCL is a multi-factorial disorder contributed to environmental and genetic factors. The genetic and environmental factors may play vital roles in the initiation and progression of DLBCL through the epigenetic modifications [4]. Several candidate gene relationship studies have shown that *TNF* is an important candidate gene for risk of DLBCL. The *TNF* gene (encoding a 233-amino acids protein), encoded by the small 4 kb gene, which is located on the short arm of chromosome 6p21.3 [5]. Several polymorphisms in the promoter region of the *TNF*-α gene have been identified. Among which, polymorphism at position -308 in the promoter region, consisting a polymorphism in the form of GG, GA and AA, has been reported to be associated with increased production of TNF-α levels both in vitro and in vivo [6, 7]. Genotype-phenotype studies of the *TNF-α* -308G/A (rs1800629) polymorphism showed the G allele conferred two-fold lower effects on the tran-

scription level when compared with the A allele [8]. Some studies in vitro have indicated that homozygosity for the A allele is associated with stronger transcription activity compared to the GG genotype, many other studies have examined the involvement of that polymorphism with both inflammation status and human complex diseases, showing that the polymorphism is positively associated with higher protein levels and an augmented risk of DLBCL [9, 10].

The TNF-α cytokine product, which is mainly produced by monocytes and macrophages, plays an important role in cell signaling and inflammation, being strongly involved in immune responses and apoptosis [11]. A mutation could affect the binding of transcription factors, resulting in altered mRNA or protein levels, a variant of a guanine by an adenine at promoter region -308 (G308A) of TNF-α has received a particularly large number of attention due to its putative effect on TNF-α expression [12]. *TNF-α* -308G/A has been widely studied because the A allele is associated with increment in transcriptional activity and increased TNF-α production [5]. Numerous studies in various countries have been performed to examine the association between the *TNF-α* 308G/A polymorphism and the risk of DLBCL and both negative and positive associations have been reported [1, 13] comparison of incidence patterns by disease subtype may provide critical clues for future etiologic investigations. We therefore conducted a comprehensive assessment of 114,548 lymphoid neoplasms diagnosed during 1992-2001 in 12 Surveillance, Epidemiology, and End Results SEER.

In the present study, we aimed to assess whether the *TNF-α* G308A gene variant is associated with DLBCL in the Chinese population and to evaluate the contribution of *TNF*-α G308A gene variant to DLBCL risk using results from different ethnic and countries by conducting meta-analysis.

Methods

Ethical approval of the research protocol

This study was approved by the Ethics Committee of First Affiliated Hospital of Xinxiang Medical University, Huazhong University of Science and Technology. Written informed consent forms were obtained from all the participants.

Subject population

In the subsequent genotyping study, we included two independent DLBCL patient case-control panels. Panel I included 245 DLBCL patient cases (admitted to the First Affiliated Hospital of Xinxiang Medical College between July 2003 and November 2014) and 245 controls (randomly selected from 1,063 individuals participating in the same regions during the same period, age and gender-matched to cases). Panel II included 306 patients (recruited from the affiliated hospital of Tongji Medical College, between January 2005 and February 2013) and 306 controls (enrolled from other healthy population in the same area during the same period). All subjects were genetically unrelated ethnic Han Chinese who provided demographic data by interview.

SNP genotyping

Genomic DNA was extracted from peripheral blood sample using QIAamp DNA Blood mini kit (Qiagen, Germany) and diluted to 10 ng/μl with AE buffer according to the manufacturer's instructions. The *TNF* 308G/A was genotyped by using the TaqMan allelic discrimination assays (Applied Biosystems, USA) [13]. Approximately 5% of the samples were repeated and the concordance was 100%.

Statistical analysis

Sample size was estimated by Quanto version 1.2.4. Comparison of clinical variables between DLBCL cases and healthy controls were performed using Student's t-test and are represented as mean ± SD. The allelic and genotypic frequencies were compared between patients and control subjects, and the Hardy-Weinberg equilibrium (P>0.05) in controls before the analysis was calculated by using Chi squared test. Logistic regression analyses were conducted to evaluate independent associations between the *TNF* 308G/A polymorphisms and DLBCL as measured by odds ratio (OR) and corresponding 95% confidence interval (CI) after adjusting for multiply covariates including KPS (Karnofsky performance status), B symptom, GCB (germinal center B cell), stage, LDH (lactate dehydrogenase), chemotherapy, IPI (international prognostic index), bone marrow involvement. The statistical analysis was done using SPSS version 16 (SPSS, Chicago, Illinois, USA). For all analyses, two-sided *P*-values less than 0.05 were considered statistically significant.

Table 1. Distribution of selected characteristics among case patients and control subjects

	Wuhan Panel (panel I)				Shanghai and Nanjing Panel (panel II)			
	Cases		Controls		Cases		Controls	
	(n=245)		(n=245)		(n=306)		(n=306)	
Characteristic	No.	%		P	No.	%		P
Age, years				0.119				0.211
<60	115	46.9	118		147	48	145	
≥60	130	53.1	127		159	52	161	
Sex				0.148				0.163
Male	137	55.9	129		177	57.8	162	
Female	108	44.1	116		129	42.2	144	
Karnofsky performance status								
80% or more	19	7.8			25	82.2		
Less than 80%	226	92.2			281	91.8		
B symptom								
Yes	125	51			168	54.9		
No	120	49			138	45.1		
GCB								
Yes	83	33.9			107	35		
No	162	66.1			199	65		
Ann Arbor stage								
1	37	15.1			58	19		
2	34	13.9			34	11.1		
3	93	38			67	21.9		
4	81	33.1			147	48		
LDH								
≤ Normal	108	44.1			138	45.1		
> Normal	137	55.9			168	54.9		
Initial chemotherapy								
CHOP	92	37.6			92	30.1		
R+CHOP	153	62.4			214	69.9		
IPI								
0	39	15.9			52	17		
1	56	22.9			64	20.9		
2	50	20.4			55	18		
3	48	19.6			57	18.6		
4	49	20			76	24.8		
5	3	1.2			2	0.7		
Bone marrow involvement						100		
Yes	41	16.7			46	15.4		
No	204	83.3			259	84.6		

Literature search strategy

Electronic databases (PubMed, Web of Science, Embase and Cochrane) were searched up to June 2015 to identify the relevant studies on the association between *TNF* 308G/ polymorphisms and risk of DLBCL in humans with the search terms of "*TNF* 308G/A", "polymorphism", in combination with "DLBCL" without language restriction. In addition, hand searching was also conducted and no other limits were employed.

Study selection

The selected studies should meet the following criteria: (1) a case control or cohort study design to measure the association between

TNF-α -308G/A and DLBCL risk; (2) containing integrated information about allele or genotype frequency for risk estimates, and providing OR with corresponding 95% CI or sufficient information to assess them; (3) original studies of animal studies, reviews, commentaries and case reports were excluded. If subjects were overlapped in several studies, only the one with more complete design or larger sample size was selected.

Data extraction

For each study, the following information including first author, the year of publication, country, and ethnicity of the study population, study location, total number of cases and controls, and the allele frequency of the participants were also extracted.

Statistical analysis

Meta-analysis was performed with STATA 12.0 (STATA Corporation, College Station, TX, USA). All associations were presented as OR with the corresponding 95% CI. The association between the *TNF* 308G/A polymorphism and DLBCL susceptibility was assessed under the following genetic models, which were treated as a dichotomous variable: (a) A-allele versus G-allele for allele level comparison; (b) AA/AG versus GG for a dominant model; (c) AA versus AG/GG for a recessive model, and (d) AA versus GG model; (e) A allele vs. G allele model. Statistical heterogeneity was measured using Q statistics and I^2 test [14]. A random effects model was used when the effects were assumed to be homogenous (P<0.10), otherwise, the fixed-effect model was used to evaluate the summary ORs and 95% CIs [15-17] In addition, sensitivity analysis was used to assess the influence of each study on the overall estimate after removal of every study [17] methodology for the meta-analysis of these studies has been neglected, particularly with regard to two issues: testing Hardy-Weinberg equilibrium (HWE. Finally, the Begg's funnel plot and Egger's test were performed to analyze the publication bias [18].

Results

Characteristics of study population

A total of 551 subjects were included in this case-control study of panel I and panel II, including 245 patients with DLBCL and 245 healthy controls in panel I from Xinxiang Medical College, and 306 patients with DLBCL and 306 healthy controls in panel II from Union Hospital. The characteristics of the study subjects were summarized in **Table 1**. Because of the matching, there were no significant deviation was observed in distributions of age, sex, and family DLBCL history between the cases and controls both in the test set and the validation set (*P*>0.05 for all).

Association of TNF 308G/A polymorphism and DLBCL

As presented in **Table 2**, in panel I, logistic regression analysis revealed that subjects with the 308AA genotypes had a 1.67-fold (*P*=0.013), while the combined -308GA/GA genotypes a 1.65-fold risk of developing DLBCL (*P*=0.069), respectively; 308GA had no significantly increased DLBCL risk (*P*=0.072), when compared with the 308GG genotype.

In panel II, subjects with 308AA, or 308GA/GA, when compared with AA genotypes, also had a significantly increased risk of DLBCL (adjusted odds ratio [OR], 1.76, and 1.63, respectively; all *P*<0.05).

We pooled the two center participants to increase the statistical power and further for stratification analysis, based on the associations in the two independent case-control studies with homogeneous (*P*=0.13). In the pooled set of 551 DLBCL cases and 551 controls, individuals carrying 308AA genotypes had a 70% increased risk of DLBCL, carrying 308GA/AA had a 65% increased risk of DLBCL, compared to the 308GG genotype (OR=1.77, 95% CI=1.31-2.51, *P*=0.01, and OR=1.65, 95% CI=1.15-1.98, *P*=0.057, respectively).

Overall meta-analysis of the association between TNF G/A and DBCLC risk

Literature search and characteristics of eligible studies: As shown in **Figure 1**, 75 potentially relevant literatures were identified and screened, of which, 9 articles met the inclusion criteria in this meta-analysis. Finally, combining the current study, a total of 10 publications [13, 19-26]. Canadian, and US case-control studies of the International Lymphoma Epidemiology Consortium (InterLymph comprising 5,199 cases and 15,470 controls were included in the

Table 2. Genotype frequencies of tnf 308g/a among case patients and control subjects and their associations with risk of DLBCL in two Chinese panels

	Case		Control			
TNF Genotypes by Panel	(No=245)	%	(No=306)	%	OR	*P* value
Discovery set (Panel I)						
GG	143	58.4	218	89	1.00 (reference)	
GA	90	36.7	25	10.2	1.13 (0.95-1.49)	0.072
AA	12	4.9	2	0.8	1.67 (1.25-1.81)	0.013
GA/AA	102	41.6	27	11	1.65 (1.12-1.58)	0.069
Trend test *P* value						0.024
Validation set (Panel II)						
GG	175	57.2	277	90.5	1.00 (reference)	
GA	103	33.7	27	8.8	1.58 (0.99-2.13)	0.019
AA	28	9.2	1	0.3	1.76 (1.29-2.47)	0.004
GA/AA	131	42.8	28	9.2	1.63 (1.14-2.24)	0.012
Trend test *P* value						0.008
Pool set						
GG	318	57.7	495	89.8	1.00 (reference)	
GA	193	35	52	9.4	1.30 (0.98-1.58)	0.022
AA	40	7.3	4	0.7	1.77 (1.31-2.51)	0.01
GA/AA	243	44.1	56	10.2	1.65 (1.15-1.98)	0.057
Trend test *P* value						0.031

Abbreviation: OR, odds ratio; Data were calculated by unconditional logistic regression, adjusted for KPS, B symptom, GCB, stage; LDH, chemotherapy regimen, IPI, bone marrow involvement.

meta-analysis (**Table 3**). Of these, 8 studies were conducted in Caucasians and 2 in Asian. Genotypes of *TNF-α* G308A in controls conformed to Hardy-Weinberg equilibrium except the study conducted by Rothman ($P<0.01$). The studies were published between 2006 and 2013 and were conducted in various populations of different ethnicities: three American [1, 13, 22] comparison of incidence patterns by disease subtype may provide critical clues for future etiologic investigations. We therefore conducted a comprehensive assessment of 114,548 lymphoid neoplasms diagnosed during 1992-2001 in 12 Surveillance, Epidemiology, and End Results (SEER, one Norway [26] p<0.001, one Australian [20] and one Sweden [23].

Overall meta-analysis of the association between TNF-α 308G/A and DLBCL risk

The evaluations of the association of *TNF* 308G/A polymorphism with DLBCL risk are shown in **Table 4** and **Figure 2**. Overall, we found that the variant AG/AA homozygote was associated with a significantly increased risk of DLBCL (AA versus GG: OR=1.79, 95% CI=1.24-2.59, $P_{heterogeneity}$=0.01; AA vs. AG: OR=1.37, 95% CI=1.13-1.65, $P_{heterogeneity}$=0.29; AA versus G carrier: OR=1.68, 95% CI=1.21-2.33, $P_{heterogeneity}$=0.007, A vs. G: OR=1.34, 95% CI=1.03-1.75, $P_{heterogeneity}$<0.001).

Subgroup analysis

When stratified according to ethnicity, we found the A allele carriers had a significantly increased risk of DLBCL among Caucasians (AA versus GG: OR=1.63, 95% CI=1.37-1.96, $P_{heterogeneity}$= 0.56; AA vs. AG: OR=1.31, 95% CI=1.10-1.56, $P_{heterogeneity}$=0.41; AA versus G carrier: OR=1.56, 95% CI=1.30-1.87, $P_{heterogeneity}$=0.59), but null result found among Asians except our study. Further stratified analysis by country, significantly elevated DLBCL risks were found for the Americans (AA versus GG: OR=1.81, 95% CI=1.31-2.50, $P_{heterogeneity}$=0.49; AA vs. AG: OR=1.41, 95% CI=1.03-1.93, $P_{heterogeneity}$=0.97; AA versus G carrier: OR=1.74, 95% CI=1.25-2.42, $P_{heterogeneity}$=0.60).

Moreover, when stratifying by genotyping methods, significantly increased cancer risks were observed for TaqMan method (AA versus GG:

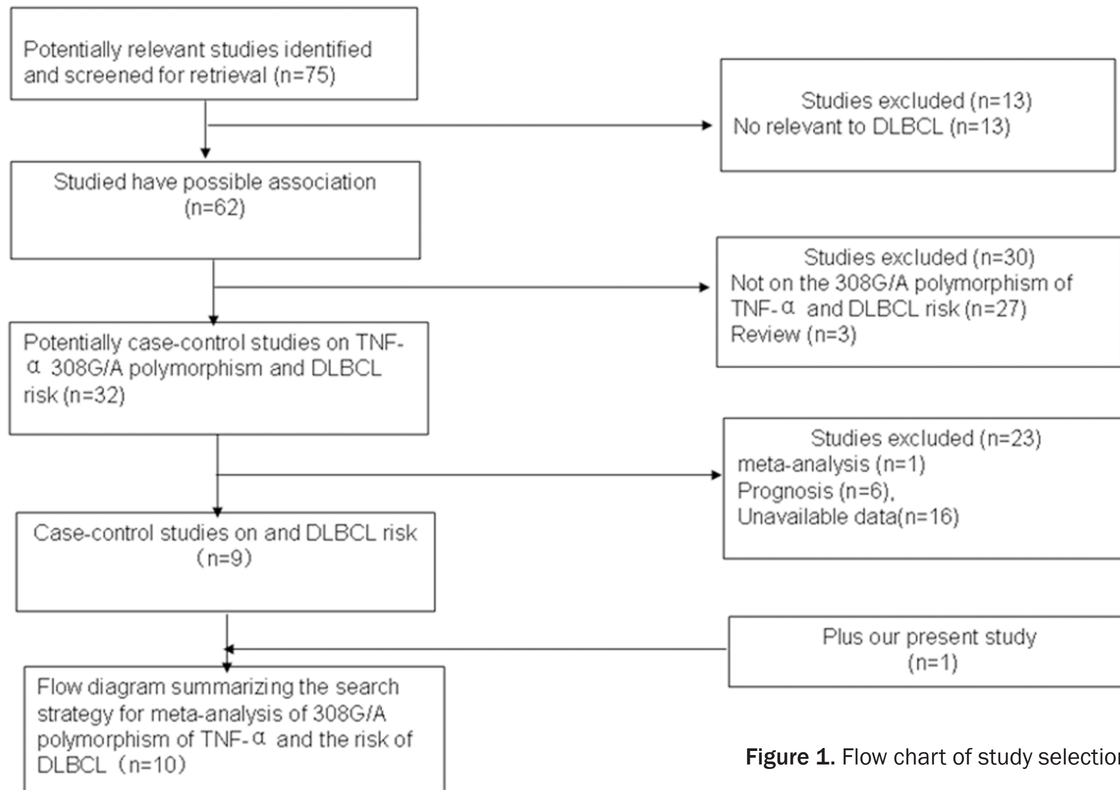

Figure 1. Flow chart of study selection.

Table 3. General characteristics of studies included in the meta-analysis

First Author	Year	Ethnicity	Country	Resource of controls	Genotyping methods	Case			Control			HWE[b]	MAF
						GG	GA	AA	GG	GA	AA		
Rothman	2006	Caucasians	mix	PCC[a]	TaqMan	716	312	53	2597	854	113	NA	0.38
Purdue	2007	Caucasians	Australia	PCC	TaqMan	106	59	5	318	143	21	0.34	0.34
Cerhan	2008	Caucasians	America	HCC[a]	Multiplex PCR	49	16	4	304	117	18	0.12	0.41
Morton	2008	Caucasians	America	PCC	TaqMan	236	102	16	696	207	18	0.57	0.38
Thunberg	2010	Caucasians	Sweden	HCC	Multiplex PCR	119	30	16	158	69	11	0.33	0.46
Fernberg	2010	Caucasians	Swiss	PCC	Multiplex PCR	371	173	23	1007	431	46	0.99	0.37
Skibola	2010	Caucasians	America	PCC	TaqMan	543	258	32	2297	858	79	0.92	0.36
Hosgood	2013	Asian	Mix	HCC	TaqMan	829	97	3	3091	506	25	0.39	0.45
Yri	2013	Caucasians	Norway	PCC	Multiplex PCR	275	178	27	664	279	32	0.69	0.34

a, HCC, hospital based case-control studies; PCC, population based case-control studies; b, HWE, Hardy-Weinberg equilibrium.

OR=1.88, 95% CI=1.01-3.4, $P_{heterogeneity}$<0.001; AA versus AG: OR=1.27, 95% CI=1.03-1.56, $P_{heterogeneity}$=0.29), and Multiply PCR method (AA versus GG: OR=1.62, 95% CI=1.20-2.18, $P_{heterogeneity}$=0.71; AA versus AG: OR=1.42, 95% CI=1.07-1.90, $P_{heterogeneity}$=0.27, AA versus AG: OR=1.42, 95% CI=1.07-1.90, $P_{heterogeneity}$=0.27, AA vs. G carriers: OR=1.57, 95% CI=1.17-2.13, $P_{heterogeneity}$=0.71).

Interestingly, when stratifying by source of controls, a significantly increased risk was found among population-based studies (AA versus GG: OR=2.02, 95% CI=1.33-3.08, $P_{heterogeneity}$< 0.001; AA vs. AG: OR=1.23, 95% CI=1.10-1.55, $P_{heterogeneity}$=0.56; AA versus G carrier: OR=1.80, 95% CI=1.24-2.60, $P_{heterogeneity}$=0.01), but not among hospital-based studies. Limiting the analysis to the studies within HWE, the results were persistent and robust (see **Table 4**).

Sensitivity analysis

In the sensitivity analysis, the influence of the individual dataset on the pooled ORs was inves-

Table 4. Subgroup analyses for the associations between TNF-α 308G/A and risks of DLBCL

Indexes		No. of studies	AA vs. GG			AA vs. GA			AA vs. G carriers			A carriers vs. GG			A vs. G		
			OR (95% CI)[a]	P_{beg}[b]	I^{2c}	OR (95% CI)[a]	P_{her}	I^2	OR (95% CI)[a]	P_{her}	I^2	OR (95% CI)[a]	P_{her}	I^2	OR (95% CI)[a]	P_{her}	I^2
All		10	1.79 (1.24-2.59)	0.01	68.1	1.37 (1.13-1.65)	0.29	16.3	1.68 (1.21-2.33)	0.007	60.5	1.35 (0.99-1.82)	<0.001	93.4	1.34 (1.03-1.75)	<0.001	93.6
Ethnicity	Caucasians	8	1.63 (1.37-1.96)	0.56	0	1.31 (1.10-1.56)	0.36	8.4	1.56 (1.30-1.87)	0.58	0	1.27 (1.12-1.45)	0.04	53.1	1.27 (1.15-1.40)	0.09	42.7
	Asians	2	2.67 (0.08-7.18)	<0.001	94.8	1.34 (0.32-5.59)	0.08	67.9	2.27 (0.10-9.44)	<0.001	93.4	2.13 (0.24-8.80)	<0.001	99.2	2.00 (0.26-5.65)	<0.001	99.2
Country	US	3	1.81 (1.31-2.50)	0.5	0	1.41 (1.03-1.93)	0.8	0	1.74 (1.25-2.42)	0.6	0	1.22 (1.12-1.34)	0.23	31.9	1.26 (1.45-1.39)	0.21	36.1
	Other	7	1.47 (1.19-1.82)	0.14	40.1	1.23 (1.00-1.51)	0.14	39.6	1.41 (1.14-1.74)	0.17	36.1	1.09 (0.84-1.41)	<0.001	85.7	1.10 (0.89-1.37)	<0.001	85.1
Design	HCC[d]	3	1.16 (0.69-1.95)	0.35	10.7	1.61 (0.60-4.31)	0.65	0	1.26 (0.75-2.13)	0.42	0	0.78 (0.67-0.91)	0.21	29.9	0.81 (0.70-0.95)	0.13	42.0
	PCC[d]	7	2.02 (1.33-3.08)	<0.001	74.0	1.23 (1.10-1.55)	0.56	0	1.80 (1.24-2.60)	<0.001	66.7	1.66 (1.20-2.29)	<0.001	93.5	1.58 (1.19-2.12)	<0.001	94
methods	TaqMan	6	1.88 (1.01-3.4)	<0.001	81.3	1.27 (1.03-1.56)	0.29	18.6	1.70 (0.98-2.95)	<0.001	76.7	1.55 (0.99-2.44)	<0.001	96	0.49 (0.99-2.24)	<0.001	96.2
	Multiply PCR	4	1.62 (1.20-2.18)	0.71	0	1.42 (1.07-1.90)	0.27	22.9	1.57 (1.17-2.13)	0.71	2.0	1.59 (0.82-1.51)	0.01	74	1.18 (0.96-1.45)	0.06	60

a, OR (95% CI), odds ratio (95% confidence interval); b, P_{value} for Heterogeneity, if P<0.10, random effects model was used, otherwise, fixed effects model was used. I square calculated by %; c, I^2 was the abbreviations of I square (%); d, HCC and PCC were the abbreviations of hospital-based case-control study and population-based case-control study.

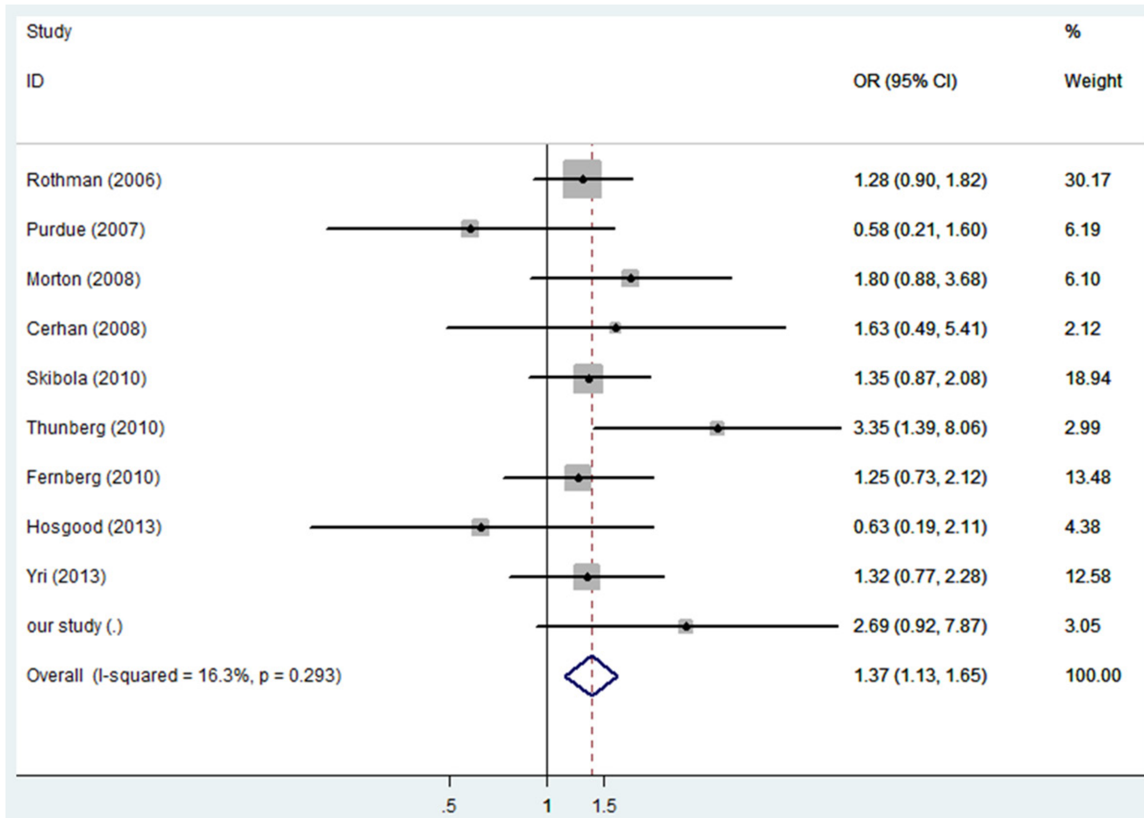

Figure 2. Forest plot showed that individuals carrying the 308A allele have an increased risk of DLBCL (AA vs. AG. Fixed effects model was used).

tigated by repeating the meta-analysis while omitting each study. This procedure proved that our results were reliable and robust. In addition, the estimated pooled OR still did not change at all when excluding the studies that were not in HWE.

Publication bias evaluation

Funnel plot was generated to evaluate the potential publication bias. Begg's rank correlation method [27] and Egger's weighted regression method [18] were used to provide statistical evidence of funnel plot asymmetry. No publication bias was detected (AA vs. GG: Begg's test *P*=0.72, Egger's test *P*=0.93; AA vs. AG: Begg's test *P*=0.72, Egger's test *P*=0.77; AA vs. G carrier: Begg's test P=1.00, Egger's test *P*=0.90; A carrier vs. GG: Begg's test *P*=0.59, Egger's test *P*=0.86; A allele vs. G allele Begg's test *P*=0.86, Egger's test *P*=0.82) (shown in **Figure 3**).

Discussion

Tumor necrosis factor alpha may play a role in the pathophysiology of DLBCL [28]. To the best of our knowledge, this is the first molecular epidemiological study to investigate the associations of *TNF-α* 308G/A polymorphism with DLBCL risk in two center Chinese Han population and first meta-analysis to assess the association between *TNF-α* G308A polymorphism with DLBCL risk. Our case-control study including the discovery set, validation set and pooled set, showed that *TNF-α* G308A polymorphism was significantly associated with an increased risk of DLBCL in Chinese Han population. Further meta-analysis indicated that *TNF-α* G308A polymorphism may influence DLBCL risk.

Accumulating studies shows that *TNF-α* G308A polymorphism strongly correlates with TNF level in serum [29]. Lower serum level was observed in participants carrying GG genotype, intermediate in GA genotype, higher in AA genotype and in both cases and control subjects. No significant difference was observed when comparing the difference in median level of *TNF* between cases and controls using nonparametric Mann-Whitney test. Our findings are con-

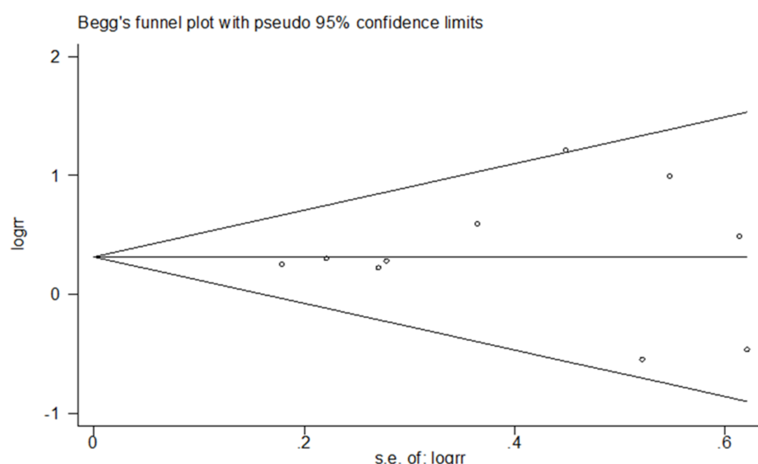

Figure 3. Begg's funnel plot for publication bias test (AA versus AG). Each point represents a separate study for the indicated association. LogOR, natural logarithm of OR.

sistent with findings of other published studies [13, 19, 21, 26]. Canadian, and US case-control studies of the International Lymphoma Epidemiology Consortium InterLymph.

The mechanism through which *TNF-α* G308A polymorphism affects the risk of DLBCL is wholly unclear. Increasing evidence has shown that, initially identified as tumor-suppressing factor, TNF-α, also involved in tumorigenesis by activating the transcription factor NF-κB, which further stimulates tumor cells. Additionally, The G to A transition in the promoter region at position -308 results in higher expression levels of *TNF-α* [30]. Homozygotes for the A allele have higher plasma *TNF* levels than carriers homozygous for the G allele [31] since cytokine production capacity varies among individuals and depends on cytokine gene polymorphisms. The association between cytokine gene polymorphisms with primary lung carcinoma was investigated. DNA samples were obtained from a Turkish population of 44 patients with primary lung cancer, and 59 healthy control subjects. All genotyping (IFN-gamma, TGF-beta1, TNF-αlpha, IL-6 and IL-10, which is critical for TNF-α's concentration-dependent activity. Currently, the *TNF-α* G308A has been extensively investigated the potential association with DLBCL risk in many studies. However, the results are incompatible. Consistent with our study, Morton et al. and Yri et al. reported an increased risk association of *TNF-α* G308A polymorphisms with DLBCL in American and Norway Caucasians [26] p<0.001, although several other studies found the null association with risk of DLBCL in the Australia population [20], the Sweden [23], and the American Caucasians [13, 21, 26].

Recently, genome-wide association studies (GWAS), which are not contingent on prior information concerning candidate gene, have made great progress in exploring the underlying genetic susceptibility to DLBCL. The GWAS result from Cerhan shows that among candidate gene studies investigating, susceptibility to DLBCL, only one locus, the LTA252G/TNF-308A haplotype on chromosome 6p21, reached genome-wide significance (P=2.9×10-8). In small GWAS of all NHL subtypes combined, no conclusive loci for DLBCL were identified in individuals of European background [32]. The opposite findings among different studies may be due to different genetic backgrounds, and environmental factors.

In the study conducted by Hosgood et al. the cases were mainly from in mixed Asian populations with DLBCL, which may confer the contribution of *TNF-α* G308A to DLBCL [25]. It is noteworthy that the minor allele frequencies among studies are different. The A allele for *TNF-α* 308G/A had a frequency of 0.34 in Australian participtants [20] and Norway [26] p<0.001, and 0.46 in DBCLC Sweden [23], and 0.18, 0.21 in DLBCL Chinese of the present study. The genetic predisposition to DLBCL in different populations may be related to the differences in allele frequencies. In our present case control study, although the sample size may not be the optimal, our power calculation for assessment set, validation set and pooled set suggests that our study has the power of 78.4%, 85.1% and 86.7% to detect a relative risk at a significant level of 0.05 which should be sufficient to describe a tendency to assess the susceptibility to DLBCL with *TNF-α* -308G/A.

In addition, the meta-analysis comprising 10 studies, plus our present study, showed that the *TNF-α* -308G/A polymorphism confers a

significant increased DLBCL risk. The pooled results indicated that there were obvious associations between *TNF-α* -308G/A polymorphism and DLBCL in under the models: allele contrast (A vs. G), homozygote (AA vs. GG), heterozygote (AG vs. GG), and recessive (AA vs. G carrier) model. Thus, the *TNF-α* -308G/A polymorphism could be suggested as a DLBCL risk factor.

When stratified by the ethnicity or nationality, significant association was observed in Caucasian but not Asian. Also, significant association was observed in American population but not the pooled populations except American. The results indicated that the genetic effect of the *TNF-α* -308G/A may harbor an ethnic specificity and nationality specificity. And this may be why some reports did not find significant association between the variant and DLBCL risk. Our findings suggested that *TNF-α* -308G/A variations had a significant increased risk of DLBCL. It also was associated with DLBCL risk in Chinese. These results were in accordance with previous studies, and further supported our findings.

The present study has some limitations that should be considered. First, as with all meta-analyses, publication bias may have occurred because it is difficult to assess the extent of unpublished data although an extensive literature search was performed. Second, more accurate analysis could be conducted if individual original data would have been available because results of our meta analysis were based on partially unadjusted estimates. Thirdly, lack of the raw data of the individual study limited any potential evaluation of potential gene-gene and gene-environment interactions.

In summary, we concluded that, current study in two center Chinese population suggested that the distribution of *TNF-α* -308G/A differed between DLBCL patients and controls. Our study and meta-analysis showed *TNF-α* -308G/A is associated with the risk of DLBCL patients. Further epidemiological and mechanistic studies on *TNF-α* -308G/A and the risk of DLBCL are warranted.

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Disclosure of conflict of interest

None.

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