Meta-analysis of the TNF-α-308G/A polymorphism and vitiligo risk

G. Nie[1,2], J.H. Qi[2], C.W. Huang[1], T. Yang[1], N. Shi[1] and Y.J. Chen[1]

[1]Department of Dermatology, Huangshi Central Hospital,
Affiliated Hospital of Hubei Polytechnic University, Huangshi, China
[2]Department of Hubei Key Laboratory of Kidney Disease Pathogenesis and Intervention, Huangshi Central Hospital,
Affiliated Hospital of Hubei Polytechnic University, Huangshi, China

Corresponding authors: N. Shi / Y.J. Chen
E-mail: 635653672@qq.com / 171703926@qq.com

Genet. Mol. Res. 14 (4): 17296-17304 (2015)
Received August 25, 2015
Accepted October 16, 2015
Published December 16, 2015
DOI http://dx.doi.org/10.4238/2015.December.16.30

ABSTRACT. Several case-control studies have been conducted to investigate the association between the tumor necrosis factor-α (TNF-α)-308G/A polymorphism and vitiligo risk. However, the results of these studies are inconsistent; therefore, we attempted to comprehensively evaluate the association between TNF-α-308G/A polymorphism and vitiligo risk via a meta-analysis. Studies reporting the association between TNF-α-308G/A polymorphism and vitiligo risk were retrieved from PubMed and EmBase databases. Data were extracted from these studies and the pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the association. Six case-control studies including 1391 vitiligo cases and 2455 control subjects were included in this meta-analysis. The overall results showed the lack of a significant difference in TNF-α-308G/A genotype distribution between the patients and controls when the G allele and GG, GG + GA, GG, and GG genotypes were compared against the A allele and the GA + AA, AA, AA, and GA genotypes, respectively (ORs = 0.65, 0.53, 0.63, 0.41, 0.55; 95%CI = 0.35-1.23, 0.24-1.18, 0.10-4.09, 0.08-1.97, 0.25-1.21; P = 0.188, 0.121, 0.627, 0.264, 0.135, respectively). This

meta-analysis suggests that the TNF-α-308G/A polymorphism may not be associated with vitiligo risk. As few studies are available in this field and current evidence remains limited, these results must be corroborated with well-designed and larger studies in the future.

Key words: Meta-analysis; Tumor necrosis factor-α; Polymorphism; Vitiligo

INTRODUCTION

Vitiligo is an acquired depigmenting skin disorder affecting approximately 0.5% of the world population (Taieb and Picardo, 2009). Loss of functional melanocytes from the epidermis is the pathological hallmark of vitiligo. The etiopathogenesis of vitiligo is far from clear, but it has been associated with autoimmune processes (Bolognia and Pawelek, 1988; Ortonne and Bose, 1993; Kovacs, 1998). Tumor necrosis factor (TNF)-α is a pleiotropic immunomodulator and pro-inflammatory cytokine that has been implicated in the pathogenesis of various kinds of autoimmune and inflammatory diseases, such as psoriasis, lepromatous leprosy, and systemic lupus erythematosus (Eigler et al., 1997; Roy et al., 1997; Sullivan et al., 1997; Kim et al., 2003). Autoimmune disorders have been proposed as the possible pathogenesis of vitiligo, leading to an increasing number of studies focusing on the relationship between TNF-α expression and incidence of vitiligo. Previous *in vitro* studies have shown that TNF-α inhibits melanogenesis and promotes melanocyte apoptosis (Swope et al., 1991; Kim et al., 2007). Several recent studies have shown the presence of increased tissue and serum levels of pro-inflammatory soluble mediators, including TNF-α, in vitiligo patients (Yu et al., 1997; Moretti et al., 2002; Tu et al., 2003). Cases of vitiligo treated with TNF-α inhibitors, albeit achieving mixed results, have also been reported (Rigopoulos et al., 2007; Simon and Burgos-Vargas, 2008; Campanati et al., 2010).

The promoter region of the TNF-α gene develops a number of polymorphisms (Allen, 1999; Brown, 2008). The most common polymorphism occurs at position -308 (TNF-α-308G/A), which may affect the cytokine production (Wilson et al., 1997; Louis et al., 1998; Abraham and Kroeger, 1999). A number of case-control studies have been conducted to investigate the association between the TNF-α-308G/A polymorphism and vitiligo risk (Yazici et al., 2006; Namian et al., 2009; Laddha et al., 2012; Salinas-Santander et al., 2012; Al-Harthi et al., 2013; Aydingoz et al., 2015). However, the results of these studies have been inconsistent, which may be attributed to small sample sizes and the differences in ethnic populations. Therefore, in this study, we performed a meta-analysis to increase the statistical power of all eligible case-control analyses, and to obtain a precise estimate of the association between the TNF-α-308G/A polymorphism and vitiligo risk.

MATERIAL AND METHODS

Literature search

Studies reporting the association between TNF-α-308G/A polymorphism and vitiligo risk published up to March 31, 2015, were independently searched without language restrictions by two of the authors in the PubMed and EmBase databases, using the following keywords: “TNF-α” or “tumor necrosis factor-α”; “vitiligo”; and “polymorphism”, “SNP”, “single nucleotide polymorphism”, “variation”, or “mutation”. The bibliographies of retrieved articles were then manually searched to find additional relevant studies.

Study selection

Studies meeting the following inclusion criteria were included in this meta-analysis: (a) evaluation of the TNF-α-308G/A polymorphism and vitiligo risk; (b) case-control studies; (c) availability of 95% confidence intervals (CIs) for odds ratios (ORs) (or possibility of calculation of the Cis); and (d) distribution of genotypes in the control group consistent with the Hardy-Weinberg equilibrium (HWE). Studies were excluded upon conformance with any of the following exclusion criteria: (a) reviews and abstracts; (b) genotype distributions not reported; and (c) family studies. If several articles were published by the same authors using the same patient data, only the study with the largest sample size or the most recent study was included.

Data extraction

The following data were independently extracted from the included studies by two of the authors: first author's name, publication date, population, sample size, genotyping method, genotype frequencies among the vitiligo patients and control subjects, and the p value of Hardy-Weinberg equilibrium (HWE) among the controls. Differences in opinion were resolved by discussion between the authors.

Statistical analysis

Genotype distributions in the controls were tested for HWE using Pearson's χ^2-test (Schaid and Jacobsen, 1999). Inter-study heterogeneity was analyzed using the Cochran Q-statistic and the I^2 test (Higgins and Thompson, 2002; Zintzaras and Ioannidis, 2005). A random-effects model was used when $P < 0.1$ for the Q-test or $I^2 > 50\%$, indicating heterogeneity; otherwise, a fixed-effects model was applied. ORs with corresponding 95%CIs were calculated to assess the association between the TNF-α-308G/A polymorphism and vitiligo risk in five genetic models (G allele *vs* A allele, GG *vs* GA + AA, GG + GA *vs* AA, GG *vs* AA, and GG *vs* GA). The significance of pooled ORs was determined using the Z-test. Begg's funnel plots and Egger's tests were used to determine the possible effect of a publication bias on the validity of the estimates; a p-value < 0.10 was considered to be significant (Peters et al., 2006). All the statistical tests were conducted using STATA 12.0 software (StataCorp LP 4905 Lakeway Drive College Station, TX, USA).

RESULTS

Characteristics of included studies

The database search helped retrieve 32 potentially relevant studies from the PubMed and EmBase databases. In accordance with the inclusion criteria, six case-control studies comprising 1391 psoriasis cases and 2455 healthy controls were included in this meta-analysis. The distribution of genotypes in the control group of each included study was consistent with the HWE (all $P > 0.05$). Two investigators independently extracted the information from eligible studies, and a consensus was reached (Table 1).

Table 1. Key information of the six studies included.

Author	Year	Ethnicity	Cases					Controls					
			Genotype			Allele		Genotype			Allele		
			GG	GA	AA	G	A	GG	GA	AA	G	A	PHWE
Yazici et al.	2006	Turkish	50	10	1	110	12	107	16	0	230	16	0.44
Namian et al.	2009	Iranian	152	17	7	321	31	470	73	2	1013	77	0.64
Salinas-Santander et al.	2012	Mexican	177	21	0	375	21	356	36	3	748	42	0.06
Laddha et al.	2012	Indian	317	311	100	945	511	780	184	17	1744	218	0.11
Al-Harthi et al.	2013	Saudi Arabian	17	103	3	137	109	100	76	24	276	124	0.11
Aydingoz et al.	2014	Turkish	93	12	0	198	12	181	27	3	389	33	0.10

PHWE, P value of the Hardy-Weinberg equilibrium (HWE).

Results of the meta-analysis

Significant inter-study heterogeneity was observed in all of the genetic models with the Q-test and the I^2 test (P < 0.1 or I^2 > 50%). Therefore, the random effects model was used to pool the results. The meta-analysis results identified the absence of any significant differences in the TNF-α-308G/A genotype distribution between the vitiligo patients and control subjects in the comparisons of the G allele *vs* the A allele, GG *vs* GA + AA, GG + GA *vs* AA, GG *vs* AA, and GG *vs* GA (OR = 0.65, 95%CI = 0.35-1.23, P = 0.188; OR = 0.53, 95%CI = 0.24-1.18, P = 0.121; OR = 0.63, 95%CI = 0.10-4.09, P = 0.627; OR = 0.41, 95%CI = 0.08-1.97, P = 0.264; and OR = 0.55, 95%CI = 0.25-1.21, P = 0.135, respectively; Figures 1-5). The results of these analyses are summarized in Table 2.

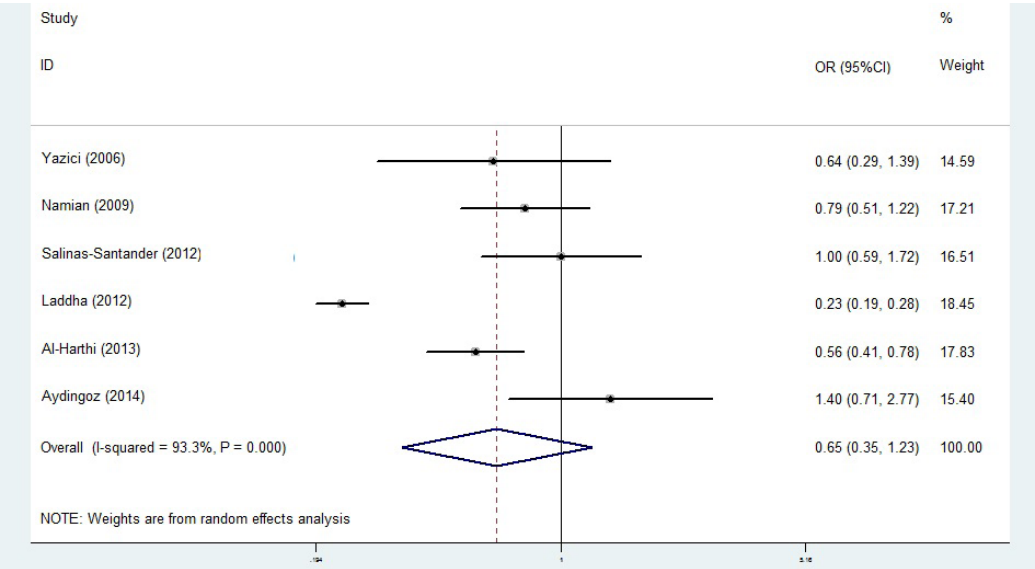

Figure 1. Forest plot depicting meta-analysis of the association between increased risk of vitiligo and TNF-α-308G/A polymorphism in an allelic model (G allele *vs* A allele), performed using a random-effects model.

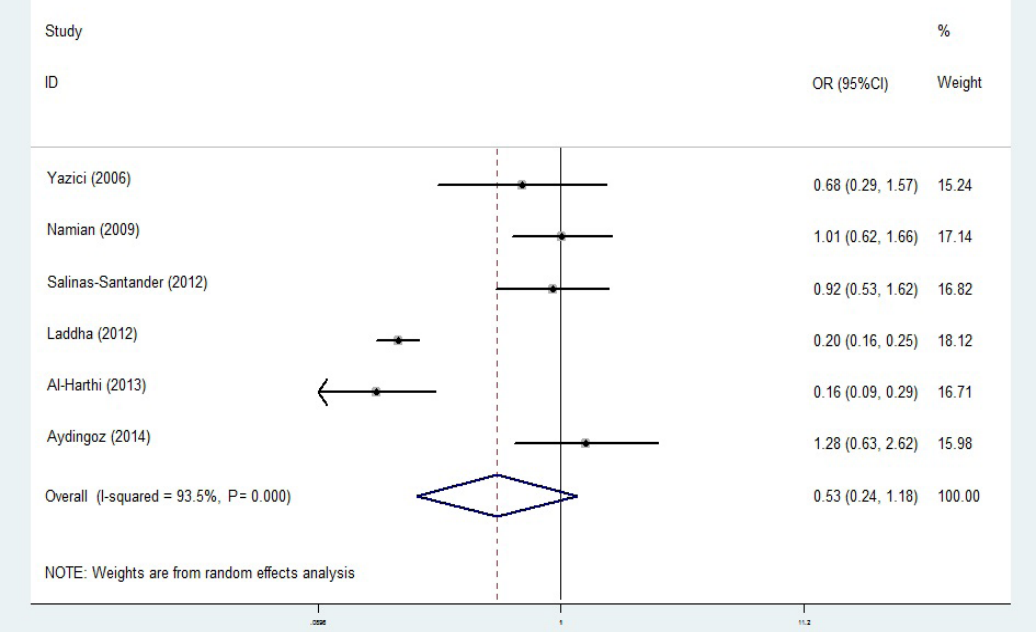

Figure 2. Forest plot depicting the meta-analysis of the association between vitiligo risk and TNF-α-308G/A polymorphism in a recessive model (GG *vs* GA + AA), conducted using a random-effects model.

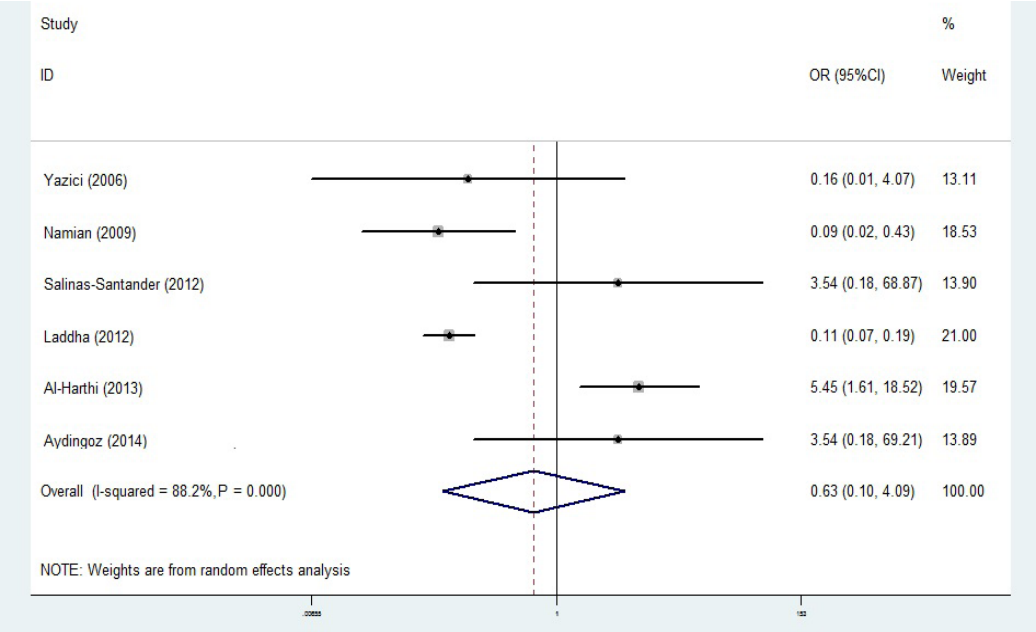

Figure 3. Forest plot depicting the meta-analysis of the association between vitiligo risk and TNF-α-308G/A polymorphism in a dominant model (GG + GA *vs* AA), performed using a random-effects model.

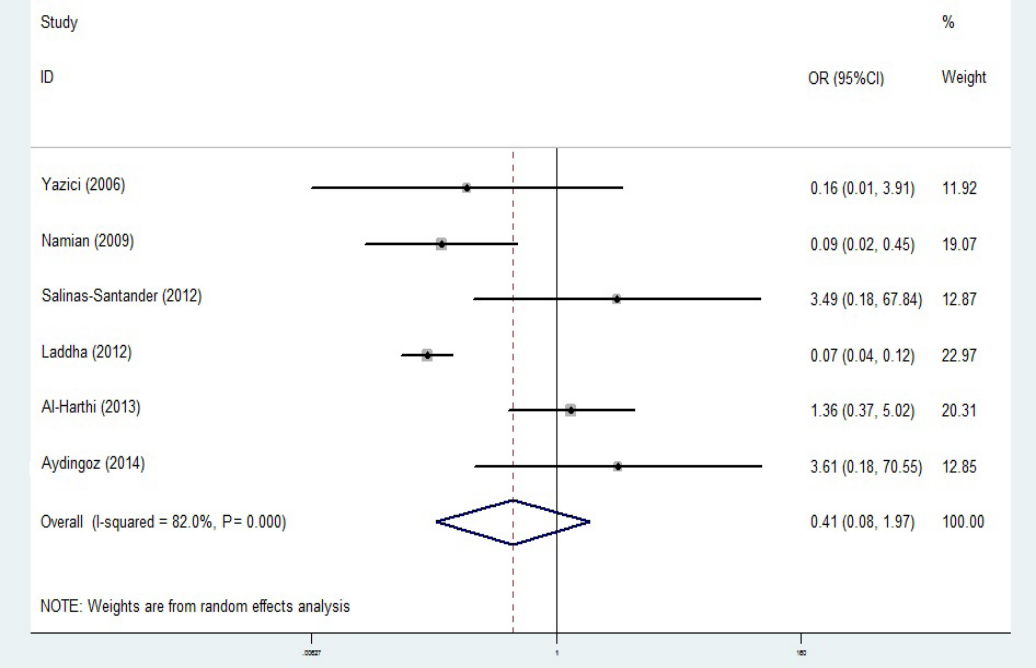

Figure 4. Forest plot depicting the meta-analysis (performed using a random-effects model) of the association between vitiligo risk and TNF-α-308G/A polymorphism in a homozygote comparison model (GG *vs* AA).

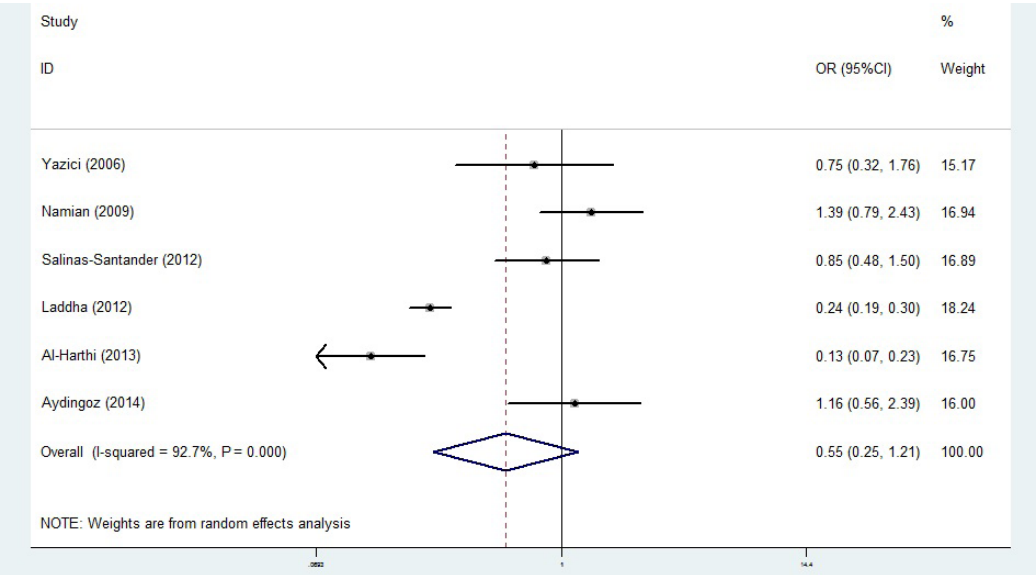

Figure 5. Forest plot depicting the meta-analysis (conducted with a random-effects model) of the association between vitiligo risk and TNF-α-308G/A polymorphism in a heterozygous comparison model (GG *vs* GA).

Table 2. Meta-analysis of the tumor necrosis factor (TNF)-α-308 G/A polymorphism and vitiligo risk.

	Sample size		Random-effects model			Test of heterogeneity	
Polymorphism	Case	Control	OR (95%CI)	Z	P	I^2 (%)	P
G allele *vs* A allele	1391	2455	0.65 (0.35-1.23)	1.32	0.188	93.3	<0.001
GG *vs* GA + AA	1391	2455	0.53 (0.24-1.18)	1.55	0.121	93.5	<0.001
GG + GA *vs* AA	1391	2455	0.63 (0.10-4.09)	0.49	0.627	88.2	<0.001
GG *vs* AA	917	2043	0.41 (0.08-1.97)	1.12	0.264	82.0	<0.001
GG *vs* GA	1280	2406	0.55 (0.25-1.21)	1.49	0.135	92.7	<0.001

OR, Odds ratio; CI, confidence interval.

Publication bias

Begg's funnel plots and Egger's tests were used to assess the publication bias. As shown in Table 3, most of the P values of Begg's funnel plots and Egger's tests were >0.10. However, the p values of the Egger's tests for G allele *vs* A allele and GG *vs* GA + AA were <0.10, indicating the presence of a significant publication bias in this meta-analysis.

Table 3. Results of the Begg's and Egger's tests.

Model	Begg's test (P)	Egger's test (P)
G allele *vs* A allele	0.260	0.013
GG *vs* GA + AA	1.000	0.097
GG + GA *vs* AA	1.000	0.276
GG *vs* AA	0.707	0.109
GG *vs* GA	1.000	0.176

DISCUSSION

The TNF-α gene is located within the class III region of the gene coding for the major histocompatibility complex (MHC) on chromosome 6 (6p21.3) (Carroll et al., 1987); several single nucleotide polymorphisms (SNPs) have been identified in the promoter region (-162, -237, -238, -274, -308, -375, -575, -850, -857, and -863) of this gene (Allen, 1999; Brown, 2008). Among these, a common polymorphism at position 308 (TNF-α-308G/A) has been studied intensively. The polymorphism appeared to influence TNF-α expression by interfering with the transcription factor binding sites or other regulatory elements; therefore, this polymorphism has been considered as a functional polymorphism (Wilson et al., 1992). To date, several studies have been carried out to identify the potential role of TNF-α-308G/A polymorphism in increased vitiligo susceptibility. However, these studies have reported conflicting results. Some studies found a significant association between the TNF-α-308G/A polymorphism and vitiligo, whereas others failed to obtain any such association. Meta-analysis has been recognized as a useful statistical method that combines findings from independent studies to precisely evaluate the effect of selected genetic polymorphisms on the risk of disease (Attia et al., 2003). To the best of our knowledge, no meta-analysis has been conducted to evaluate the association between the TNF-α-308G/A polymorphism and vitiligo risk. Therefore, there is a need to conduct a meta-analysis using published data to clarify the inconsistent findings in this field of study.

All studies included in this meta-analysis only analyzed genotypes for quality control. The genotype distribution of controls in all studies was consistent with the HWE. The results of

this meta-analysis, performed using six case-control studies including 1391 vitiligo cases and 2455 healthy controls, revealed the lack of a significant difference in the TNF-α-308G/A genotype distribution between vitiligo patients and healthy controls, when the G allele and GG, GG + GA, GG, and GG genotypes were compared against the A allele and the GA+ AA, AA, AA, and GA genotypes, respectively. The results showed obvious heterogeneity between the studies, suggesting a possible role of ethnic and environmental differences in the genetic backgrounds. Some limitations of our meta-analysis should be acknowledged. First, some relevant studies could not be included in our analysis because of lack of complete raw data or publications. Second, the number of studies included was not sufficiently large and the sample size of some of the studies included was relatively small, which may result in insufficient statistical power to explore the real association between TNF-α-308G/A polymorphism and vitiligo risk. Third, the results of the Egger tests revealed the possibility of a significant publication bias in this meta-analysis. Moreover, vitiligo may also be modulated by several other genetic markers other than TNF-α, such as *CYP2C9* (Alzolibani et al., 2014), *PTPN22* (Garcia-Melendez et al., 2014), and *ACE* (Badran et al., 2015). Therefore, the results of our meta-analysis emphasize the need for further evaluation of the potential gene-gene interactions in vitiligo, in order to elucidate the pathogenesis of vitiligo. Finally, some factors, such as the gender, age, and the clinical classification of the disease, were not considered when analyzing the data because of a lack of raw data, which may cause serious confounding bias. However, the advantages of this meta-analysis were also obvious. First, the sample size of our study was larger compared to that of the individual studies, which increased the reliability of the results. Second, the associations between TNF-α-308G/A polymorphism and vitiligo were evaluated under different genetic models.

In summary, this meta-analysis suggests that the TNF-α-308G/A polymorphism may not be associated with vitiligo risk. As few studies are available in this field and current evidence remains limited, further studies must be performed to warrant and validate our results.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Abraham LJ and Kroeger KM (1999). Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J. Leukoc. Biol.* 66: 562-566.

Al-Harthi F, Zouman A, Arfin M, Tariq M, et al. (2013). Tumor necrosis factor-alpha and -beta genetic polymorphisms as a risk factor in Saudi patients with vitiligo. *Gen. Mol. Res.* 12: 2196-2204.

Allen RD (1999). Polymorphism of the human TNF-alpha promoter--random variation or functional diversity? *Mol. Immunol.* 36: 1017-1027.

Alzolibani AA, Al Robaee A, Al-Shobaili H, Al-Saif F, et al. (2014). Association of CYP2C9 Genetic Variants with Vitiligo. *Ann. Dermatol.* 26: 343-348.

Attia J, Thakkinstian A and D'Este C (2003). Meta-analyses of molecular association studies: methodologic lessons for genetic epidemiology. *J. Clin. Epidemiol.* 56: 297-303.

Aydingoz IE, Kanmaz-Ozer M, Gedikbasi A, Vural P, et al. (2015). The combination of tumour necrosis factor-alpha -308A and interleukin-10 -1082G gene polymorphisms and increased serum levels of related cytokines: susceptibility to vitiligo. *Clin. Exp. Dermatol.* 40: 71-77.

Badran DI, Nada H and Hassan R (2015). Association of angiotensin-converting enzyme ACE gene polymorphism with ACE activity and susceptibility to vitiligo in Egyptian population. *Genet. Test. Mol. Biomarkers* 19: 258-263.

Bolognia JL and Pawelek JM (1988). Biology of hypopigmentation. *J. Am. Acad. Dermatol.* 19: 217-255.

Brown MA (2008). Re: Zhu et al. A novel gene variation of TNF alpha associated with ankylosing spondylitis: a reconfirmed study. *Ann. Rheum. Dis.* 67: 434; discussion 434-436.

Campanati A, Giuliodori K, Ganzetti G, Liberati G, et al. (2010). A patient with psoriasis and vitiligo treated with etanercept. *Am. J. Clin. Dermatol.* 11: 46-48.

Carroll MC, Katzman P, Alicot EM, Koller BH, et al. (1987). Linkage map of the human major histocompatibility complex including the tumor necrosis factor genes. *Proc. Natl. Acad. Sci. U.S.A.* 84: 8535-8539.

Eigler A, Sinha B, Hartmann G and Endres S (1997). Taming TNF: strategies to restrain this proinflammatory cytokine. *Immunol. Today* 18: 487-492.

Garcia-Melendez ME, Salinas-Santander M, Sanchez-Dominguez C, Gonzalez-Cardenas H, et al. (2014). Protein tyrosine phosphatase PTPN22 +1858C/T polymorphism is associated with active vitiligo. *Exp. Ther. Med.* 8: 1433-1437.

Higgins JP and Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21: 1539-1558.

Kim NH, Jeon S, Lee HJ and Lee AY (2007). Impaired PI3K/Akt activation-mediated NF-kappaB inactivation under elevated TNF-alpha is more vulnerable to apoptosis in vitiliginous keratinocytes. *J. Invest. Dermatol.* 127: 2612-2617.

Kim TG, Pyo CW, Hur SS, Kim YK, et al. (2003) Polymorphisms of tumor necrosis factor (TNF) alpha and beta genes in Korean patients with psoriasis. *Arch. Dermatol. Res.* 295: 8-13.

Kovacs SO (1998). Vitiligo. *Am. Acad. Dermatol.* 38: 647-666; quiz 667-648.

Laddha NC, Dwivedi M and Begum R (2012). Increased tumor necrosis factor (TNF)-alpha and its promoter polymorphisms correlate with disease progression and higher susceptibility towards vitiligo. *PLoS One* 7: e52298.

Louis E, Franchimont D, Piron A, Gevaert Y, et al. (1998). Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin. Exp. Immunol.* 113: 401-406.

Moretti S, Spallanzani A, Amato L, Hautmann G, et al. (2002). New insights into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. *Pigment Cell Res.* 15: 87-92.

Namian AM, Shahbaz S, Salmanpoor R, Namazi MR, et al. (2009). Association of interferon-gamma and tumor necrosis factor alpha polymorphisms with susceptibility to vitiligo in Iranian patients. *Arch. Dermatol. Res.* 301: 21-25.

Ortonne JP and Bose SK (1993). Vitiligo: where do we stand? *Pigment Cell Res.* 6: 61-72.

Peters JL, Sutton AJ, Jones DR, Abrams KR, et al. (2006). Comparison of two methods to detect publication bias in meta-analysis. *JAMA* 295: 676-680.

Rigopoulos D, Gregoriou S, Larios G, Moustou E, et al. (2007). Etanercept in the treatment of vitiligo. *Dermatology* 215: 84-85.

Roy S, McGuire W, Mascie-Taylor CG, Saha B, et al. (1997). Tumor necrosis factor promoter polymorphism and susceptibility to lepromatous leprosy. *J. Infect. Dis.* 176: 530-532.

Salinas-Santander M, Diaz-Garcia D, Rojas-Martinez A, Cantu-Salinas C, et al. (2012). Tumor necrosis factor-alpha -308G/A polymorphism is associated with active vitiligo vulgaris in a northeastern Mexican population. *Exp.* Ther. *Med.* 3: 893-897.

Schaid DJ and Jacobsen SJ (1999). Biased tests of association: comparisons of allele frequencies when departing from Hardy-Weinberg proportions. *Am. J. Epidemiol.* 149: 706-711.

Simon JA and Burgos-Vargas R (2008). Vitiligo improvement in a patient with ankylosing spondylitis treated with infliximab. *Dermatology* 216: 234-235.

Sullivan KE, Wooten C, Schmeckpeper BJ, Goldman D, et al. (1997). A promoter polymorphism of tumor necrosis factor alpha associated with systemic lupus erythematosus in African-Americans. *Arthritis Rheum.* 40: 2207-2211.

Swope VB, Abdel-Malek Z, Kassem LM and Nordlund JJ (1991). Interleukins 1 alpha and 6 and tumor necrosis factor-alpha are paracrine inhibitors of human melanocyte proliferation and melanogenesis. *J. Invest. Dermatol.* 96: 180-185.

Taieb A and Picardo M (2009). Clinical practice. Vitiligo. *N. Engl. J. Med.* 360: 160-169.

Tu CX, Gu JS and Lin XR (2003). Increased interleukin-6 and granulocyte-macrophage colony stimulating factor levels in the sera of patients with non-segmental vitiligo. *J. Dermatol. Sci.* 31: 73-78.

Wilson AG, di Giovine FS, Blakemore AI and Duff GW (1992). Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum. Mol. Genet.* 1: 353.

Wilson AG, Symons JA, McDowell TL, McDevitt HO, et al. (1997). Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc. Natl. Acad. Sci. U.S.A.* 94: 3195-3199.

Yazici AC, Erdal ME, Kaya TI, Ikizoglu G, et al. (2006). Lack of association with TNF-alpha-308 promoter polymorphism in patients with vitiligo. *Arch. Dermatol. Res.* 298: 46-49.

Yu HS, Chang KL, Yu CL, Li HF, et al. (1997). Alterations in IL-6, IL-8, GM-CSF, TNF-alpha, and IFN-gamma release by peripheral mononuclear cells in patients with active vitiligo. *J. Invest. Dermatol.* 108: 527-529.

Zintzaras E and Ioannidis JP (2005). Heterogeneity testing in meta-analysis of genome searches. *Gen. Epidemiol.* 28: 123-137.