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# A meta-analysis of association between acne vulgaris and *Demodex* infestation<sup>\*</sup>

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**Abstract:** Until now, etiology of acne vulgaris is still uncertain. Although clinicians usually deny the association between *Demodex* infestation and acne vulgaris, it has been proved in some clinical practices. To confirm the association between *Demodex* infestation and acne vulgaris, a meta-analysis was conducted. Predefined selection criteria were applied to search all published papers that analyzed the association between *Demodex* infestation and acne vulgaris (January 1950 to August 2011) in ISI Web of Knowledge, MEDLINE, and China National Knowledge Infrastructure (CNKI) databases. A meta-analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs) based on fixed effects models or random effects models. We enrolled the 60 Chinese and 3 English papers in this meta-analysis, which covered Turkey and 25 different provinces/municipalities in China and 42130 participants including students and residents, aged from 1 to 78 years. The pooled OR in random effects models is 2.80 (95% CI, 2.34–3.36). Stability is robust according to sensitivity analysis. The fail-safe number is 18 477, suggesting that at least 18 477 articles with negative conclusions would be needed to reverse the conclusion that acne vulgaris was related to *Demodex* infestation. So the effect of publication bias was insignificant and could be ignored. It was concluded that acne vulgaris is associated with *Demodex* infestation. This indicates that when regular treatments for acne vulgaris are ineffective, examination of *Demodex* mites and necessary acaricidal therapies should be considered.

Key words:Demodex infestation, Acne vulgaris, Case-control study, Meta-analysisdoi:10.1631/jzus.B1100285Document code: ACLC number: R384.4; R757.3

#### 1 Introduction

The *Demodex* species are microscopic, obligate, elongated mites belonging to the family Demodicidae of the order Acari of the class Arachnida. *Demodex folliculorum* and *Demodex brevis* are found parasitizing on the human body surface. *D. folliculorum* occupies the hair follicles, upper sebaceous glands level, whilst *D. brevis* exists principally in the depth of sebaceous glands. Human nose, cheeks, forehead, temples, chin, external ear tract, scalp, eyelid, and upper part of the chest are the predilection sites, where large and numerous sebaceous glands provide a favorable habitat for the mites. Though the pathogenicity of Demodex mites is still debatable, more and more case-control studies showed that the multiplication of the mites is usually considered as a cause of multiple skin disorders. They have been reported to be involved in pityriasis folliculorum (Ayres, 1930), rosacea (Ayres and Ayres, 1961; Bonnar et al., 1993; Forton and Seys, 1993), pustular folliculitis (Dong and Duncan, 2006), papulopustular scalp eruptions (Purcell et al., 1986), perioral dermatitis (Hsu et al., 2009), and blepharitis (Post and Juhlin, 1963; Divani et al., 2009; Zhao et al., 2012). It is also suspected that Demodex infestation may be one of the triggering factors of carcinogenesis in evelid basal cell carcinomas (Erbagci et al., 2003) and sebaceous adenoma (Dhingra et al., 2009). Moreover, the infestation rate in the immunocompromised population with leucocythemia (Damian and Rogers, 2003) or acquired immune deficiency syndrome (AIDS) (Clyti et al.,

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2005) is remarkably high and pustular eruption or seborrheic dermatitis becomes even more conspicuous.

Although the association between abovementioned skin disorders and Demodex infestation has been reported, clinical doctors usually deny the association between acne vulgaris and Demodex infestation, because of the high prevalence of Demodex in the common population. At present, since Demodex is a host-specific obligate parasite, and presently cannot be cultured in vitro so as to parasitize and further cause infection in other healthy animal hosts (Zhao et al., 2009; 2011c), the direct absolute proof of the causal relationship has not yet been established. Therefore, in order to clarify the disputable association between *Demodex* infestation and the development of acne vulgaris, we investigated the Chineseand English-language case-control studies on the association between Demodex infestation and acne vulgaris that we sifted from certain databases by using the statistical method of meta-analysis.

## 2 Materials and methods

## 2.1 Data sources

We searched in August 2011 for all case-control studies that analyzed the association between Demodex infestation and acne vulgaris, which were published after January 1950, in two English databases and one Chinese database: Institute for Scientific Information (ISI) Web of Knowledge (including Science Citation Index (SCI), Index to Scientific & Technical Proceedings (ISTP), Journal Citation Reports (JCR), BIOSIS Previews (BP), Information Services for Physics, Electronic Computer (INSPEC), and Derwent Innovations Index (DII)), MEDLINE, and China National Knowledge Infrastructure (CNKI). The search terms used were "Demodex". The selected articles were scanned for potentially relevant articles. The inclusion and exclusion of articles were processed by two researchers (Li HU and Li-ping WU) based on the same criteria. For disputed articles, a third researcher (Ya-e ZHAO) mediated whether or not to include.

## 2.2 Data inclusion and exclusion

We evaluated each study to determine whether to include it or not based on the following criteria:

(1) studies with "infection/infestation rate" as the study parameter; (2) studies with appropriate detection methods, including skin surface biopsy (SSB), cellophane tape preparation (CTP) method, and skin pressurization method (SPM); (3) studies with clearly and accurately reported data so that  $2\times2$  table in  $\chi^2$  test could be obtained or calculated. Papers that appeared in multiple databases were counted only once. Reviews, systematic evaluations, studies without controls, studies which did not provide sources of cases and controls, and republished papers were excluded.

## 2.3 Data analysis

For each study, multiple 2×2 tables were constructed, and  $\chi^2$  test was applied to compare *Demodex* infestation rate of cases and controls. Raw data from all the studies were pooled to establish a database, and the data were double-checked. Meta-analysis was performed using the Review Manager (version 4.2 for Windows, Oxford England, the Cochrane Collaboration, in 2002) to assess the relationship between *Demodex* infestation and acne vulgaris. The pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using random and fixed effects models, then forest plots were drawn and sensitivity and publication bias of studies were assessed.

#### 2.4 Sensitivity analysis

We compared the pooled effect sizes (1) with different statistical models, (2) of studies using the CTP, SPM, or both two methods, and (3) before and after excluding studies with small sample size, to identify the sensitivity of the studies.

#### 2.5 Publication bias identification

Publication bias is usually assessed in two ways. The funnel plot is used for qualitative study and the equation of fail-safe number for quantitative study. When there is no heterogeneity among the sifted studies, both methods are suitable to assess publication bias, whilst when heterogeneity is significant amongst the data, only equation of fail-safe number can be applied to perform quantitative analysis. Publication bias is identified according to Rosenthal (1979), who referred to 5K+10 (*K* is the number of papers included) as a parameter in file-drawer problem. When compared to 5K+10, the larger *m* is (*m* is

the least number of unpublished studies that could make pooled effect size to have no statistical significance), the less effect publication bias has on statistical results.

## 3 Results

## 3.1 Overall information

Initial searches identified 1455 English papers (929 in ISI Web of Knowledge and 526 in MEDLINE) and 1207 Chinese papers. According to the inclusion and exclusion criteria mentioned above, 60 Chinese papers covering 25 provinces/municipalities and only 3 English papers (Baysal *et al.*, 1997; Zhao *et al.*, 2011a; 2011b) were eligible, which consisted of 42130 subjects in total including students and residents, aged from 1 to 78 years. Most of the subjects were university and middle school students who were prone to acne vulgaris in the age of 11 to 25 years.

## 3.2 Meta-analysis

In single  $\chi^2$  test, 48 of the included 63 papers (Fig. 1) concluded positive association, whilst the other 15 papers found no association (Peng et al., 1983; Zhang J.S. et al., 1985; Xing et al., 1988; Li, 1990; Li et al., 1991; Chen X.N. et al., 1996; Lin, 1996; Tu'er et al., 1997; Zhan et al., 1997; Yang X.H. et al., 2001; Fang et al., 2003; Chen X.Y. et al., 2004; Chen J.F. et al., 2006; Liu and Li, 2006; Zhang H.Y. et al., 2007). Total infestation rate of Demodex mites was 51.85% (5587/10776) in acne patients, obviously higher than 31.54% (9888/31354) in the controls  $(\chi^2=1423.49, P<0.00)$ . Heterogeneity was significant among the 63 studies ( $\chi^2$ =519.09, P<0.01). Therefore, random effects model was applied to analyze the pooled data. The pooled OR was 2.80 (95% CI, 2.34–3.36). The hypothesis test of pooled effect size (P<0.05) showed that the association between De*modex* infestation and the development of acne vulgaris was statistically significant.

#### 3.3 Sensitivity analysis

As is shown in Figs. 1–5, the pooled OR was 2.80 (95% CI, 2.34–3.36) in random effects model, and 2.84 (95% CI, 2.68–3.01) in fixed effects model. The ORs of studies using the CTP, studies using SPM, and the studies using both methods were 2.95 (95%

CI, 2.32–3.75), 2.35 (95% CI, 1.37–4.01), and 2.80 (95% CI, 2.05–3.83), respectively. Six papers (Zhang J.S. *et al.*, 1985; Li, 1990; Chen Y.G. *et al.*, 1995; Zhang M.H. *et al.*, 2005; Liu and Li, 2006; Zhang H.Y. *et al.*, 2007) of the 63 included studies had sample size smaller than 40. After these articles were excluded, the pooled OR of the remaining ones was 2.84 (95% CI, 2.36–3.42). No significant difference was found amongst all these pooled ORs. So sensitivity analysis was robust, revealing that the association existed consistently between *Demodex* infestation and the development of acne vulgaris.

#### 3.4 Publication bias identification

In this study, since significant heterogeneity existed among the 63 included papers ( $\chi^2$ =519.09, P<0.01), fail-safe number formulas were used to perform the quantitative analysis of publication bias. The fail-safe number was 18477, which was significantly larger than 5*K*+10=325, suggesting that at least 18477 articles with negative conclusions would be needed to reverse the conclusion that acne vulgaris was related to *Demodex* infestation. Therefore, the effect of publication bias was insignificant and could be ignored.

# 4 Discussion

Albeit *Demodex* infestation as a risk factor for rosacea has been confirmed recently (Zhao et al., 2010), the association between acne vulgaris and Demodex infestation has not. Acne vulgaris is different from rosacea. They are two distinct pathologies. with different symptoms, physiopathologies, causes, and treatments. Acne vulgaris is a multifactorial disease, originating in the pilosebaceous unit. The main symptoms include the microcomedone, comedone, papules, pustules, nodus, cyst, and scar. Thus, according to the type and the severity of clinical lesions, acne can be divided into comedonal acne, papulopustular acne, and nodular acne. Acne can also be classified, according to the age of onset, such as neonatal acne, infantile acne, pubertal acne, and adult acne. However, until now, the etiology of acne vulgaris is still uncertain. It is currently considered as related to factors such as androgen, increasing sebum secretion, dyskeratosis of pilosebaceous duct,

Study Ac r sub-category	ne vulgaris n/N	Control n/N	OR (random) 95% Cl	VVeight %	CR (random) 95% Cl	Variar
Zhao <i>et al.</i> , 2011b	52/129	98/321	-8-	1.77	1.54 [1.01, 2.35]	0.
Zhao <i>et al.</i> , 2011a	186/227	266/477	-9-	1.81	3.60 [2.45, 5.28]	0.
rang X.H. <i>et al.</i> , 2011	34/64	28/192		1.58	6.64 [3.52, 12.51]	0.
<ong <i="">et al., 2011</ong>	26/58	96/423		1.65	2.77 [1.57, 4.87]	0.
Nong <i>et al.</i> , 2011	39/64	53/217		1.63	4.83 [2.68, 8.71]	0.
rang X.H. <i>et al.</i> , 2010	39/76	74/356		1.69	4.02 [2.39, 6.74]	0.
Nang H.Y. <i>et al.</i> , 2010	51/89	137/789		1.74	6.39 [4.04, 10.10]	0.
Shang <i>et al.</i> , 2010	442/590	62/95		1.74	1.59 [1.00, 2.52]	0.
He <i>et al.</i> , 2009 Song <i>et al.</i> , 2009	20/41	66/221		1.54 1.40	2.24 [1.14, 4.40]	0.
Vang and Wang, 2008	17/108	10/186 122/809		1.88	3.29 [1.45, 7.47]	0. 0.
hen L.Q. <i>et al.</i> , 2008	26/77	52/306		1.66	6.21 [4.71, 8.20] 2.49 [1.42, 4.35]	0.
	57/113	294/1276		1.80	3.40 [2.30, 5.03]	0.
ao <i>et al.</i> , 2007		69/409		1.87		0.
)i <i>et al.</i> , 2007 hang H.Y. <i>et al</i> . , 2007	398/831 18/24	123/169		1.25	4.53 [3.38, 6.07]	0.
ang H.N. <i>et al.</i> , 2006	88/106			1.55	1.12 [0.42, 3.00]	
iu and Li, 2006		28/95			11.70 [5.97, 22.91]	0.
ang and Luo, 2006	6/16	294/559		1.21	0.54 [0.19, 1.51]	0.
u et al., 2006	20/52	52/256		1.58	2.45 [1.30, 4.63]	0. 0.
hen J.F. et al., 2006	35/59	1168/2947			2.22 [1.31, 3.75]	
	12/45	95/576		1.52	1.84 [0.92, 3.69]	0.
hang M.H. <i>et al</i> ., 200: in X.H. <i>et al</i> ., 2005		8/30		1.05	11.46 [3.44, 38.18]	0.
	94/344	44/287			2.08 [1.39, 3.10]	0.
iu <i>et al.</i> , 2005	50/82	38/186		1.65	6.09 [3.44, 10.75]	0.
uo et al., 2005	28/97	30/167		1.63	1.85 [1.03, 3.35]	0.
ao G.L. et al., 2005	65/90	188/665		1.72	6.60 [4.04, 10.78]	0.
ou <i>et al.</i> , 2004	154/205	389/2059		1.84	12.96 [9.27, 18.13]	0.
hao et al., 2004	46/72	44/107		1.60	2.53 [1.37, 4.69]	0.
hen X.Y. <i>et al.</i> , 2004 hou and Wu, 2003	64/115	20/49	-	1.54	1.82 [0.92, 3.59]	0.
	90/166	332/794		1.84	1.65 [1.18, 2.31]	0.
iang <i>et al.</i> , 2003	224/401	153/324		1.87	1.41 [1.05, 1.90]	0.
et al., 2003	47/91	131/820	-a-	1.75	5.62 [3.58, 8.83]	0.
ang <i>et al.</i> , 2003	20/139	21/192		1.56	1.37 [0.71, 2.64]	0.
hoù <i>et al.</i> , 2002 iang <i>et al.</i> , 2002	138/178	425/1788		1.82	11.06 [7.65, 15.99]	0.
	32/101	106/547		1.74	1.93 [1.21, 3.09]	0.
hou and Zhao, 2001	46/144	27/190	-#	1.68	2.83 [1.66, 4.95]	0.
ang and Duan, 2001	54/56	250/852			65.02 [15.73, 268.72]	0.
ang X.H. <i>et al.</i> , 2001	23/43	207/499		1.59	1.62 [0.87, 3.03]	0.
uo <i>et al.</i> , 2001	57/109	66/293		1.74	3.77 [2.37, 6.00]	0.
ia <i>et al.</i> , 1999	52/148	38/300		1.73	3.73 [2.31, 6.03]	0.
ang Z.H. <i>et al.</i> , 1998	297/783	349/1860	*	1.92	2.65 [2.20, 3.18]	0.
laysal <i>et al.</i> , 1997	12/101	0/50		→ 0.34	14.11 [0.82, 243.30]	2.
han <i>et al.</i> , 1997	37/280	35/288		1.71	1.10 [0.67, 1.80]	0.
u'er <i>et al.</i> , 1997	46/65	348/500		1.65	1.06 [0.60, 1.87]	0.
.in, 1996	120/154	272/358	- <u>F</u> -	1.75	1.12 [0.71, 1.75]	0.
hen X.N. <i>et al.</i> , 1996	55/196	59/248		1.77	1.25 [0.82, 1.92]	0.
lu et al., 1995	55/207	25/200	-9-	1.69	2.53 [1.51, 4.26]	0.
hen Y.G. et al., 1995	26/32	30/108		1.25	11.27 [4.22, 30.09]	0.
long, 1994	37/153	13/150		1.54	3.36 [1.71, 6.63]	0.
lan, 1994	39/43	46/63		1.08	3.60 [1.12, 11.61]	0.
hen X.C. <i>et al.</i> , 1993	162/185	130/185		1.67	2.98 [1.74, 5.11]	0.
tu <i>et al.</i> , 1993 an and Zhang, 1992	55/207	25/200	2	1.69	2.53 [1.51, 4.26]	0.
	512/737	126/234	-8-	1.86	1.95 [1.44, 2.64]	0.
hang X.S. <i>et al.</i> , 1991 Loto(1991		13/112		1.62	7.18 [3.94, 13.08]	0.
i <i>et al.</i> , 1991	11/56	172/691		1.54	0.74 [0.37, 1.46]	0.
i, 1990	31/36	326/432		1.26	2.02 [0.76, 5.32]	0.
an, 1989	48/76	819/1587		1.73	1.61 [1.00, 2.59]	0.
ing et al., 1988	30/47	794/1439	+	1.61	1.43 [0.78, 2.62]	0.
in J. <i>et al.,</i> 1988	76/131	197/432		1.80	1.65 [1.11, 2.45]	0.
an and Tao, 1988	27/68	110/502		1.68	2.35 [1.38, 3.99]	0.
un <i>et al.</i> , 1987	108/193	161/420	-	1.83	2.04 [1.45, 2.89]	0.
hang J.S. <i>et al.</i> , 1985	0/38	37/314	← • ← ← ←	0.34	0.10 [0.01, 1.60]	2.
'eng <i>et al.</i> , 1983	335/505	76/102		1.72	0.67 [0.42, 1.09]	0.
Vang Y.P. et al., 1982	92/101	21/51		1.34	14.60 [6.04, 35.31]	0.
otal (95% Cl)	10776	31354	*	100.00	2.80 [2.34, 3.36]	
otal events: 5587 (Acne	vulgaris), 9888 (	Control)				
est for heterogeneity: est for overall effect: Z	x <sup>2</sup> = 519.09, df=	62 (P<0.00001),	• 88.1%			
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			.01 0.1 1 10	100		

 Review:
 Meta Analysis of the Association on Demodex Infestation and Acne vulgaris

 Comparison:
 01 Acne vulgaris VS. Control

 Outcome:
 01 Meta analysis of 63 included studies with acne vulgaris

Fig. 1 Forest plot of the 63 included studies about the association between *Demodex* and acne vulgaris

Review:	Meta Analysis of the Association on Demodex Infestation and Acne vulgaris
Comparison:	01 Acne vulgaris vs. control
Outcome:	02 Meta analysis of 57 included large sample studies with acne vulgaris

or sub-category Zhao et al., 2011b Zhao et al., 2011 a Yang X.H. et al., 2011 Kong et al., 2011 Nong et al., 2011 Yang X.H. et al., 2010 Wang H.Y. et al., 2010 Shang et al., 2010 He et al., 2009 Song et al., 2009	n/N 52/129 186/227 34/64 26/58 39/64 39/76 51/89 442/590	n/N 98/321 266/477 28/192 96/423 53/217	95% CI	% 1.90 1.93 1.69	95% Cl 1.54 [1.01, 2.35] 3.60 [2.45, 5.28]	Varianc 0.05 0.04
Zhao <i>et al.</i> , 2011 a Yang X.H. <i>et al.</i> , 2011 Kong <i>et al.</i> , 2011 Nong <i>et al.</i> , 2011 Yang X.H. <i>et al.</i> , 2010 Wang H.Y. <i>et al.</i> , 2010 Shang <i>et al.</i> , 2010 He <i>et al.</i> , 2009	186/227 34/64 26/58 39/64 39/76 51/89	266/477 28/192 96/423 53/217	*	1.93	3.60 [2.45, 5.28]	
Yang X.H. <i>et al.</i> , 2011 Kong <i>et al.</i> , 2011 Yang X.H. <i>et al.</i> , 2010 Wang H.Y. <i>et al.</i> , 2010 Shang <i>et al.</i> , 2010 He <i>et al.</i> , 2009	34/64 26/58 39/64 39/76 51/89	28/192 96/423 53/217				0.04
Kong <i>et al.</i> , 2011 Nong <i>et al.</i> , 2011 Yang X.H. <i>et al.</i> , 2010 Mang H.Y. <i>et al.</i> , 2010 Shang <i>et al.</i> , 2010 He <i>et al.</i> , 2009	26/58 39/64 39/76 51/89	96/423 53/217		1.69		
Nong <i>et al.</i> , 2011 (ang X.H. <i>et al.</i> , 2010 Mang H.Y. <i>et al.</i> , 2010 Shang <i>et al.</i> , 2010 He <i>et al.</i> , 2009	39/64 39/76 51/89	53/217		1.76	6.64 [3.52, 12.51]	0.10
Yang X.H. <i>et al.</i> , 2010 Wang H.Y. <i>et al.</i> , 2010 Shang <i>et al.</i> , 2010 He <i>et al.</i> , 2009	39/76 51/89			1.74	2.77 [1.57, 4.87] 4.83 [2.68, 8.71]	0.08
Nang H.Y. <i>et al.</i> , 2010 Shang <i>et al.</i> , 2010 He <i>et al.</i> , 2009	51/89	74/356		1.81	4.02 [2.39, 6.74]	0.03
Shang <i>et al.</i> , 2010 He <i>et al.</i> , 2009		137/789		1.87	6.39 [4.04, 10.10]	0.05
He <i>et al.</i> , 2009		62/95		1.86	1.59 [1.00, 2.52]	0.06
Song et al 2009	20/41	66/221		1.64	2.24 [1.14, 4.40]	0.12
bong or an 2000	17/108	10/186		1.49	3.29 [1.45, 7.47]	0.18
Nang and Wang, 2008	203/387	122/809	-	2.01	6.21 [4.71, 8.20]	0.02
Chen L.Q. <i>et al.</i> , 2008	26/77	52/306		1.77	2.49 [1.42, 4.35]	0.08
'ao <i>et al.</i> , 2007	57/113	294/1276	-8-	1.93	3.40 [2.30, 5.03]	0.04
Di et al., 2007	398/831	69/409	-	2.00	4.53 [3.38, 6.07]	0.02
ang H.N. <i>et al.</i> , 2006	88/106	28/95		1.65	11.70 [5.97, 22.91]	0.12
ang and Luo, 2006	20/52	52/256		1.69	2.45 [1.30, 4.63]	0.11
Du <i>et al.</i> , 2006 Chen J.F. et al. 2006	35/59	1168/2947		1.80	2.22 [1.31, 3.75]	0.07
Chen J.F. <i>et al.</i> , 2006	12/45	95/576		1.62	1.84 [0.92, 3.69]	0.13
Qin X.H. <i>et al.</i> , 2005 Liu <i>et al.</i> , 2005	94/344	44/287 38/186		1.92	2.08 [1.39, 3.10] 6.09 [3.44, 10.75]	0.04
Guo <i>et al.</i> , 2005	50/82	30/167		1.73	1.85 [1.03, 3.35]	0.08
ao G.L. <i>et al.</i> , 2005	28/97	188/665		1.83	6.60 [4.04, 10.78]	0.06
Lou et al., 2004	65/90 154/205	389/2059		1.97	12.96 [9.27, 18.13]	0.03
hao <i>et al.</i> , 2004	46/72	44/107		1.71	2.53 [1.37, 4.69]	0.10
hen X.Y. et al., 2004	64/115	20/49		1.64	1.82 [0.92, 3.59]	0.1
hou and Wu, 2003	90/166	332/794		1.97	1.65 [1.18, 2.31]	0.03
iang <i>et al.</i> ,2003	224/401	153/324	-8-	2.00	1.41 [1.05, 1.90]	0.0
i <i>et al.</i> , 2003	47/91	131/820		1.87	5.62 [3.58, 8.83]	0.0
ang <i>et al.</i> , 2003	20/139	21/192		1.67	1.37 [0.71, 2.64]	0.1
hou <i>et al.</i> , 2002	138/178	425/1788		1.95	11.06 [7.65, 15.99]	0.0
iang <i>et al.</i> , 2002	32/101	106/547		1.86	1.93 [1.21, 3.09]	0.00
hou and Zhao, 2001	46/144	27/190		1.79	2.83 [1.66, 4.85]	0.0
ang and Duan, 2001	54/56	250/852		• 0.94	65.02 [15.73, 268.72]	0.5
ang X.H. <i>et al.</i> , 2001	23/43	207/499		1.70	1.62 [0.87, 3.03]	0.10
Suo <i>et al.</i> , 2001 (ia <i>et al.</i> , 1999	57/109	66/293		1.86	3.77 [2.37, 6.00]	0.00
ang Z.H. et al., 1998	52/148	38/300 349/1860		1.85	3.73 [2.31, 6.03] 2.65 [2.20, 3.18]	0.0
Baysal <i>et al.</i> , 1997	297/783 12/101	0/50		→ 0.35	14.11 [0.82, 243.30]	2.11
(han <i>et al.</i> , 1997	37/280	35/288		1.83	1.10 [0.67, 1.80]	0.06
u'er <i>et al.</i> , 1997	46/65	348/500		1.76	1.06 [0.60, 1.87]	0.0
.in, 1996	120/154	272/358		1.87	1.12 [0.71, 1.75]	0.0
hen X.N. et al., 1996	55/196	59/248	- <b>-</b>	1.89	1.25 [0.82, 1.92]	0.0
u <i>et al.</i> , 1995	55/207	25/200		1.81	2.53 [1.51, 4.26]	0.0
long, 1994	37/153	13/150		1.64	3.36 [1.71, 6.63]	0.1
lan, 1994	39/43	46/63		1.14	3.60 [1.12, 11.61]	0.3
hen X.C. <i>et al.</i> , 1993	162/185	130/185		1.79	2.98 [1.74, 5.11]	0.0
u et al. 1993	55/207	25/200		1.81	2.53 [1.51, 4.26]	0.0
an and Zhang, 1992	512/737	126/234	-	2.00	1.95 [1.44, 2.64]	0.0
nang X.S. <i>et al.</i> , 1991 i <i>et al.</i> , 1991	298/614	13/112		1.73	7.18 [3.94, 13.08] 0.74 [0.37, 1.46]	0.0
	11/56	172/691 819/1587		1.64	1.61 [1.00, 2.59]	0.1
an, 1989 ing <i>et al.</i> , 1988	48/76	794/1439	-	1.72	1.43 [0.78, 2.62]	0.0
in J. et al., 1988	30/47	197/432		1.92	1.65 [1.11, 2.45]	0.0
an and Tao, 1988	76/131 27/68	110/502		1.80	2.35 [1.38, 3.99]	0.0
un <i>et al.</i> , 1987	108/193	161/420	-#-	1.96	2.04 [1.45, 2.89]	0.0
eng <i>et al.</i> , 1983	335/505	76/102		1.84	0.67 [0.42, 1.09]	0.0
/ang Y.P. <i>et al.</i> , 1982	92/101	21/51		1.42	14.60 [6.04, 35.31]	0.2
otal (95% Cl) otal events: 5481 (Acne vul			•	100.00	2.84 [2.36, 3.42]	
est for heterogeneity: $\chi^2 =$ est for overall effect: Z = 11						

Fig. 2 Forest plot of 57 studies with large sample size about the association between *Demodex* and acne vulgaris

Review:	Meta Analysis of the Association on Demodex infestation and Acne vulgaris
Comparison:	01 Acne vulgaris vs. control
Outcome:	03 Meta analysis of 36 included studies with cellophane tape preparation method

Study Acne or sub-category /			Control n/N	OR (random) 95% Cl	Weight %	OR (random) 95% Cl	Variance
Yang X.H. <i>et al.</i> , 2	011	34/64	28/192		2.75	6.64 [3.52, 12.51]	0.10
Kong et al., 2011		26/58	96/423		2.86	2.77 [1.57, 4.87]	0.08
Yang X.H. et al., 2	010	39/76	74/356		2.94	4.02 [2.39, 6.74]	0.07
Wang H.Y. et al., 2		51/89	137/789	-8	3.03	6.39 [4.04, 10.10]	0.05
He et al., 2009		20/41	66/221		2.68	2.24 [1.14, 4.40]	0.12
Song et al., 2009		17/108	10/186		2.44	3.29 [1.45, 7.47]	0.18
Wang and Wang,	2008	203/387	122/809	-57-	3.25	6.21 [4.71, 8.20]	0.02
Chen L.Q. et al., 20		26/77	52/306	-3-	2.87	2.49 [1.42, 4.35]	0.08
Di et al., 2007		398/831	69/409	-10-	3.24	4.53 [3.38, 6.07]	0.02
Zhang H.Y. et al., 2	2007	18/24	123/169		2.17	1.12 [0.42, 3.00]	0.25
Liu and Li, 2006		6/16	294/559		2.10	0.54 [0.19, 1.51]	0.27
Yang and Luo, 200	06	20/52	52/256		2.75	2.45 [1.30, 4.63]	0.11
Chen J.F. et al., 20	006	12/45	95/576		2.65	1.84 [0.92, 3.69]	0.13
Zhang M.H. et al., 2		25/31	8/30		- 1.84	11.46 [3.44, 38.18]	0.38
Qin X.H. et al., 200		94/344	44/287		3.11	2.08 [1.39, 3.10]	0.04
Liu et al., 2005	0.000	50/82	38/186		2.86	6.09 [3.44, 10.75]	0.08
Guo et al., 2005		28/97	30/167		2.82	1.85 [1.03, 3.35]	0.09
Cao G.L. <i>et al.</i> 200	06	65/90	188/665		2.98	6.60 [4.04, 10.78]	0.06
Zou <i>et al.</i> , 2004	0.5	154/205	389/2059		3.19	12.96 [9.27, 18.13]	0.03
Chen X.Y. et al 20	004	64/115	20/49	-	2.68	1.82 [0.92, 3.59]	0.12
Zhou and Wu, 200		90/166	332/794		3.19	1.65 [1.18, 2.31]	0.03
Liang et al., 2003	~	224/401	153/324		3.24	1.41 [1.05, 1.90]	0.03
Fang <i>et al.</i> , 2003			21/192		2.72	1.37 [0.71, 2.64]	0.02
liang et al., 2003		20/139	106/547		3.01	1.93 [1.21, 3.09]	0.06
Zhou and Zhao, 2002	0.04	32/101			2.91		0.08
		46/144	27/190			2.83 [1.66, 4.85]	
ang and Duan, 20		54/56	250/852	_		65.02 [15.73, 268.72]	0.52
Yang X.H. et al., 20	001	23/43	207/499	† <b>*</b>	2.77	1.62 [0.87, 3.03]	0.10
Suo <i>et al.</i> , 2001		57/109	66/293		3.02	3.77 [2.37, 6.00]	0.06
Kia <i>et al.</i> , 1999		52/148	38/300	-8	3.00	3.73 [2.31, 6.03]	0.06
Zhan <i>et al.</i> , 1997		37/280	35/288		2.97	1.10 [0.67, 1.80]	0.06
Chen X.N. et al., 19	396	55/196	59/248		3.07	1.25 [0.82, 1.92]	0.05
Hu et al., 1995		55/207	25/200		2.93	2.53 [1.51, 4.26]	0.07
Vong, 1994		37/153	13/150	6	2.68	3.36 [1.71, 6.63]	0.12
Han, 1994		39/43	46/63		1.89	3.60 [1.12, 11.61]	0.36
Chen X.C. <i>et al.</i> , 19	393	162/185	130/185		2.91	2.98 [1.74, 5.11]	0.08
Ru <i>et al.</i> , 1993		55/207	25/200		2.93	2.53 [1.51, 4.26]	0.07
Totai (95% Cl)		5410	14019	*	100.00	2.95 [2.32, 3.75]	
Total events: 2388 (/				2007			
Test for heterogeneit Test for overall effec			< 0.00001), ] <sup>2</sup> = 87.2%				
			0.01	0.1 1 10	100		

Control Acne vulgaris

#### Fig. 3 Forest plot of 36 studies about the association between Demodex and acne vulgaris using the cellophane tape preparation method

tudy r sub-category	Acne vulgaris n/N	Control n/N	OR (random) 95% Ci	Weight %	OR (random) S5% Cl	l Varianci
Zhao <i>et al.</i> , 2011b	52/129	98/321	-10-	8.73	1.54 (1.01, 2.3	35] 0.05
(ang H.N. et al., 200	6 88/106	28/95		8.06	11.70 [5.97, 22.	.91] 0.12
_in, 1996	120/154	272/358		8.66	1.12 [0.71, 1."	75] 0.05
Chen Y.G. et al., 199	5 26/32	30/108		7.05	11.27 [4.22, 30.	.09] 0.25
Zhang X.S. et al., 19		13/112	-6	8.28	7.18 [3.94, 13.	.08] 0.09
i et al., 1991	11/56	172/691		8.03	0.74 [0.37, 1.4	46] 0.12
_i, 1990	31/36	326/432		7.09	2.02 [0.76, 5.3	32] 0.24
king <i>et al.</i> , 1988	30/47	794/1439		8.26	1.43 [0.78, 2.6	52] 0.09
Qin.J. et al., 1988	76/131	197/432		8.79	1.65 [1.11, 2.4	45] 0.04
Han and Tao, 1988	27/68	110/502	-0-	8.47	2.35 [1.38, 3.9	99] 0.07
Zhang J.S. et al., 198		37/314	4 = =	2.61	0.10 (0.01, 1.0	50] 2.06
Peng et al., 1983	335/505	76/102	-9-	8.59	0.67 [0.42, 1.0	
Nang Y.P. <i>et al.</i> , 198		21/51		7.38	14.60 [6.04, 35.	.31] 0.20
		4957		100.00	2.35 (1.37, 4.0	

Control Acne vulgaris

Fig. 4 Forest plot of 13 studies about the association between *Demodex* and acne vulgaris using skin pressurization method

Study or sub-category	Acne vulgaris n/N	Control n/N	OR (random) 95% Cl	Weight %	OR (r 95	Variance	
Zhao <i>et al.</i> , 2011a	186/227	266/477		8.00	3.60 [2.4	5, 5.28]	0.04
Nong et al., 2011	39/64	53/217		6.87	4.83 [2.6	8, 8.71]	0.09
Shang et al., 2010	442/590	62/95		7.59	1.59 [1.0	2.52]	0.06
Tao <i>et al.</i> , 2007	57/113	294/1276	-8-	7.96	3.40 [2.3	, 5.03]	0.04
Du et al., 2006	35/59	1168/2947		7.24	2.22 [1.3	. 3.75]	0.07
Zhao <i>et al.</i> , 2004	46/72	44/107		6.73	2.53 [1.3	4.69]	0.10
Ji et al., 2003	47/91	131/820		7.65	5.62 [3.5	8, 8.83]	0.05
Zhou et al., 2002	138/178	425/1788		8.07	11.06 [7.6	5, 15.99]	0.04
Yang Z.H. et al., 1998	297/783	349/1860	-	8.81	2.65 [2.2	, 3.18]	0.01
Tu'er et al., 1997	46/65	348/500	-	7.00	1.06 [0.6	1.87]	0.08
Pan and Zhang, 1992	512/737	126/234	-	8.38	1.95 [1.4	4, 2.64]	0.02
Pan. 1989	48/76	819/1587		7.51	1.61 [1.0	2.59]	0.06
Sun et al., 1987	108/193	161/420	-	8.18	2.04 [1.4	5, 2.89]	0.03
Total (95% CI)	3248	12328	•	100.00	2.80 [2.0	5, 3.83]	
Total events: 2001 (Acne vu	Igaris), 4248 (Control	)					
Test for heterogeneity: $\chi^2$ =	= 100.67, df = 12 (P<	0.00001), J <sup>2</sup> = 88.1%					
Test for overall effect: $Z = 6$	.44 (P< 0.00001)						
		0.01 0	0.1 1 10	100			
			Control Acne vulgaris				

 Review:
 Meta Analysis of the Association on Demodex Infestation and Acne vulgaris

 Comparison:
 01 Acne vulgaris VS. Control

 Outcome:
 05 Meta analysis of 13 included studies with both methods

Fig. 5 Forest plot of 13 studies about the association between *Demodex* and acne vulgaris using both the cellophane tape preparation method and skin pressurization method

follicular orifice block up, proliferation of *Propionibacterium acnes*, or heredity. In recent decades, more and more clinical case-control studies have reported that *Demodex* infestation is associated with acne vulgaris, albeit much controversy persists. In the study, 48 of the included 63 papers concluded positive association, while the rest 15 papers found no association.

In spite of the significant heterogeneity among the 63 included Chinese- and English-language papers ( $\chi^2$ =519.09, P<0.01), the results of meta-analysis demonstrated statistical association between De*modex* infestation and acne vulgaris, with pooled OR 2.80 (95% CI, 2.34–3.36). The sensitivity analysis is robust. The conclusion will not be overthrown until it includes 18477 articles with negative results. Also, the detection methods we applied in the present study, namely the CTP and SPM, were the same as those used in our previous study on the association between rosacea and Demodex infestation (Zhao et al., 2010). In the study about rosacea, the analysis results obtained from different detection methods [standardized SSB (Forton and Seys, 1993; Hsu et al., 2009), skin biopsy (Moravvej et al., 2007; Hsu et al., 2009), CTP (Yao, 2005; Di et al., 2007), and SPM (Wang and Zhang, 2006; Cao Y.S. et al., 2009)] were identical. Therefore, we argue that the conclusion of our present

study and the included 63 papers, where CTP and pressurization methods were used, would be reliable.

The heterogeneity arose due to differences across studies in sample size, design or conduct, geographic region, population characteristics, and sensitivity of detection methods. Of the 63 included articles, 15 concluded no association for two possible reasons. The first one might be sample bias. In the 15 studies, 4 had sample size smaller than 40 (Zhang J.S. et al., 1985; Li, 1990; Liu and Li, 2006; Zhang H.Y. et al., 2007), and 7 had unmatched sample size in patient and control (Peng et al., 1983; Xing et al., 1988; Li et al., 1991; Tu'er et al., 1997; Yang X.H. et al., 2001; Chen X.Y. et al., 2004; Chen J.F. et al., 2006), leading to significant statistical bias. The second one might be the inadequate use of detection methods. Two of them did not apply CTP in the cheeks, but only in the nasal ala which is not flat, leading to low detection rate and missed diagnosis (Zhan et al., 1997; Fang et al., 2003). No obvious reason was found in the other two (Chen X.N. et al., 1996; Lin, 1996).

Demodex mites have been considered to be possibly related to many kinds of facial dermatoses. However, its role as a risk factor has only recently been confirmed in rosacea (Zhao *et al.*, 2010). In the present study, based on the results we have obtained, we may conclude that acne vulgaris is also

significantly related to Demodex infestation. However, it is not related as closely to Demodex infestation (OR 2.80, 95% CI, 2.34-3.36) as rosacea is (OR 7.57, 95% CI, 5.39–10.62). The possible reason is that age of onset in acne vulgaris is younger than that in rosacea, which is in negative correlation with that Demodex infestation rises with age (Divani et al., 2009; Zhao et al., 2011a). On the one hand, in acne vulgaris, as adolescents gradually age, they secrete androgen, and their sebaceous glands mature, resulting in pubertal acne. At the same time, mature sebaceous glands may increase the chances of *Demodex* infestation. The damage in the hair follicles and sebaceous glands caused by multiplication of *Demodex* mites overlaps with pubertal acne and then could aggravate the symptoms. Conversely, Demodex infestation may play a direct pathogenic role in adult acne-like demodicosis. The causative factors of pubertal acne (e.g., excess androgen secretion) are no longer present in the adults. The direct damage of hair follicles and sebaceous glands caused by excess Demodex infestation should be one reason of adult acne-like demodicosis. Here, it is worth explaining that dermatologists in China mainland rarely put the diagnosis of demodicosis in their practice, and probably the demodicoses or demodicoses with dermatoses simultaneously are today often misdiagnosed as acne (and other facial dermatoses).

Although clinicians usually deny the association between *Demodex* infestation and acne vulgaris, the association between acne-like demodicosis and Demodex infestation has been proved in some clinical practices. Yuan et al. (2003) conducted a single-blind and controlled clinical trial with 100 acne-like demodicidosis cases ( $\geq 5$  mites/cm<sup>2</sup>) to explore the acaricidal effect of volatile oil of Chinese Zanthoxylum bungeanum. The experimental group was treated with the cream made from the volatile oil of Chinese Z. bungeanum and the control group with the generally used acaricidal medicine in China, new Fumanling cream, main compositions of which are Gynocardia odorata, stemona root, fructus, hairyvein agrimonia herb, Ammi majus, arilin, etc. Before treatment, the amounts of inflammatory skin lesions in the two groups were 24.6±7.6 and 24.8±7.7, respectively, and *Demodex* densities were (12.8±3.9) and  $(12.9\pm3.9)$  mites/cm<sup>2</sup>, respectively. Differences between the two groups were insignificant (P>0.05).

After six weeks' treatment, the amounts of inflammatory skin lesions decreased to  $4.3\pm2.6$  and  $6.8\pm3.3$ , respectively, and *Demodex* densities decreased to  $(1.2\pm1.0)$  and  $(2.2\pm1.3)$  mites/cm<sup>2</sup>, respectively. There were significant differences between pre-treatment and post-treatment patients and between treatment and control groups after the treatment. It suggested that the *Demodex* density played an important role in demodicosis. Also the decrease in *Demodex* density and alleviation in clinical symptoms after acaricidal treatments provided indirect proof of association between *Demodex* infestation and acne-like demodicosis.

Acne vulgaris can affect every age group. It manifests several similar symptoms to demodicidosis, including papules, pustules, etc. This leads to the indeterminateness in clinical diagnosis of acne vulgaris and the difficulty in differential diagnosis. Only if excess Demodex was detected could we associate the lesions with *Demodex*. In the present study, although we included and analyzed 63 papers about the association between Demodex infestation and acne vulgaris, only 3 English papers (Baysal et al., 1997; Zhao et al., 2011a; 2011b) matched the inclusion criteria. Also only one study we could searched used SSB method (Okyay et al., 2006), and it did not match the inclusion criteria. The pervasiveness of the results and conclusions was impaired because of the scarcity of English articles. More convincing association between *Demodex* infestation and acne vulgaris might be obtained by future studies, in which standard skin surface biopsy (SSSB) is used to measure the mites density (Forton, 2007), and a large sample controlled study is conducted to observe the different *Demodex* density between acne vulgaris patients and control patients.

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