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A meta-analysis of association between acne vulgaris and *Demodex* infestation*

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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 42 130 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-analysis
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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 eyelid 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 above-mentioned 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 Chinese- and 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 2×2 table in χ^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 χ^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 χ^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 (χ^2=1423.49, P<0.00). Heterogeneity was significant among the 63 studies (χ^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 *Demodex* 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 (χ^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 $5K$+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,

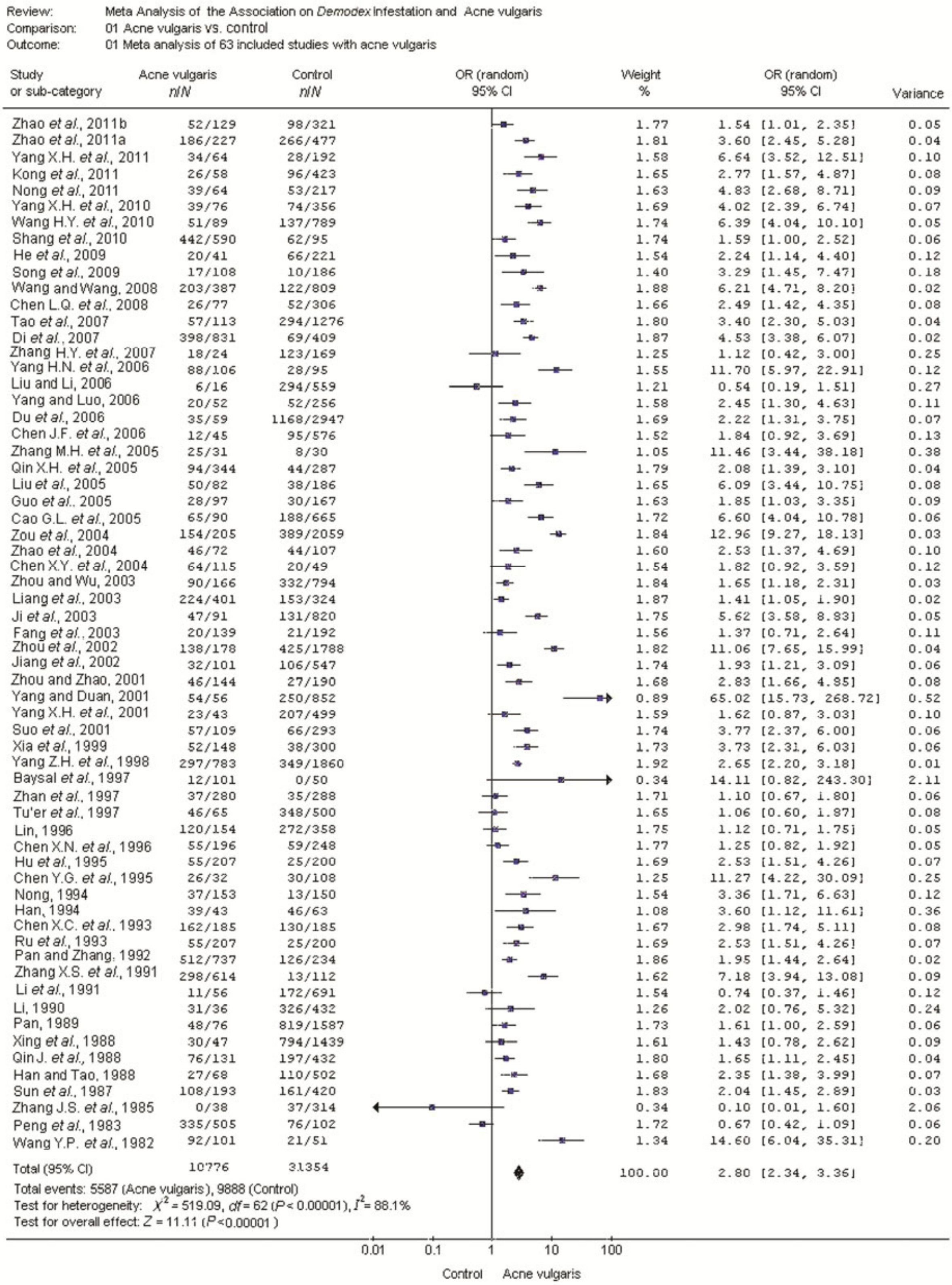

Fig. 1 Forest plot of the 63 included studies about the association between *Demodex* and acne vulgaris

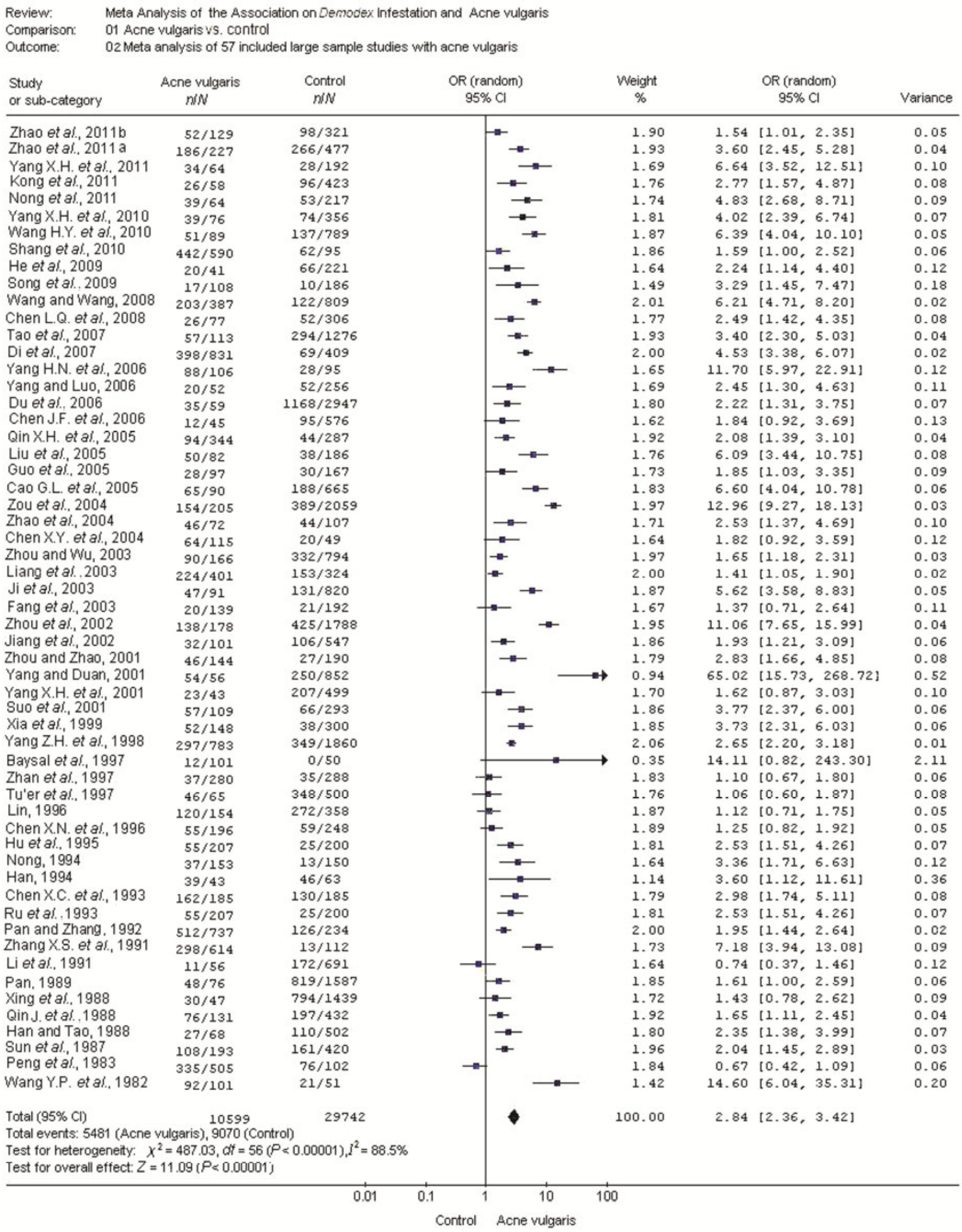

Fig. 2 Forest plot of 57 studies with large sample size about the association between *Demodex* and acne vulgaris

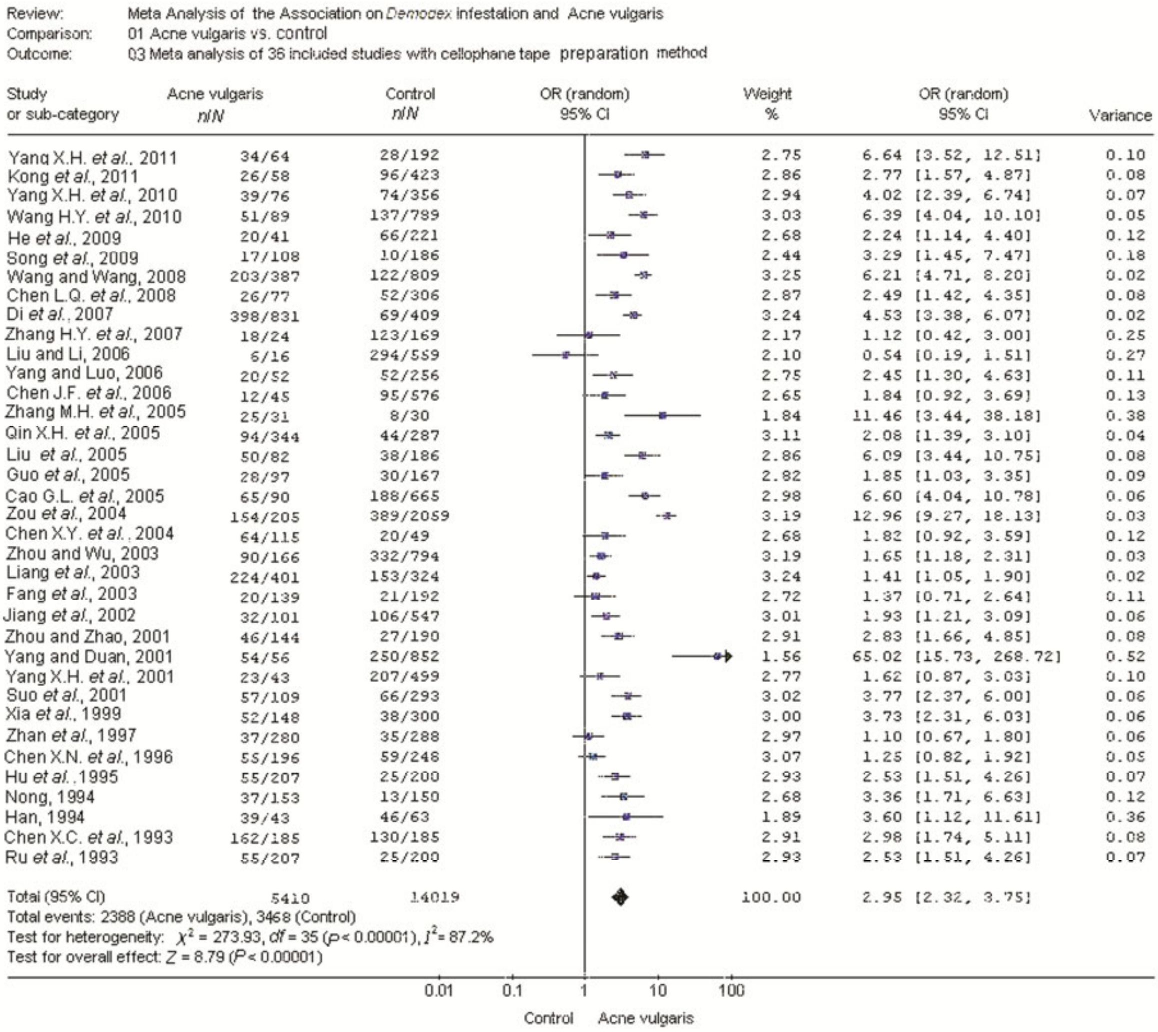

Fig. 3 Forest plot of 36 studies about the association between *Demodex* and acne vulgaris using the cellophane tape preparation method

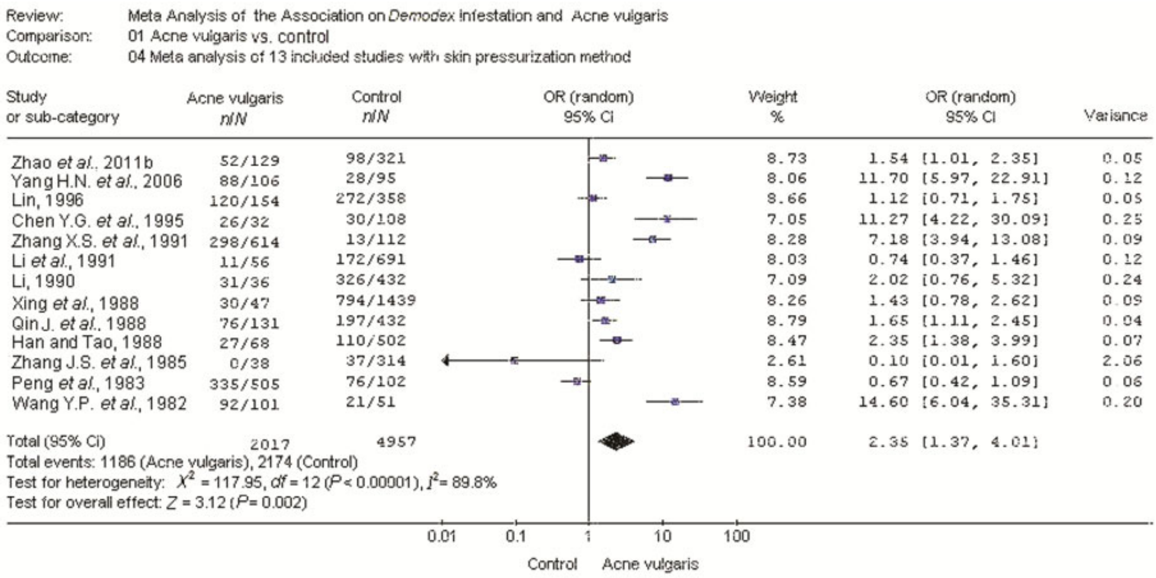

Fig. 4 Forest plot of 13 studies about the association between *Demodex* and acne vulgaris using skin pressurization method

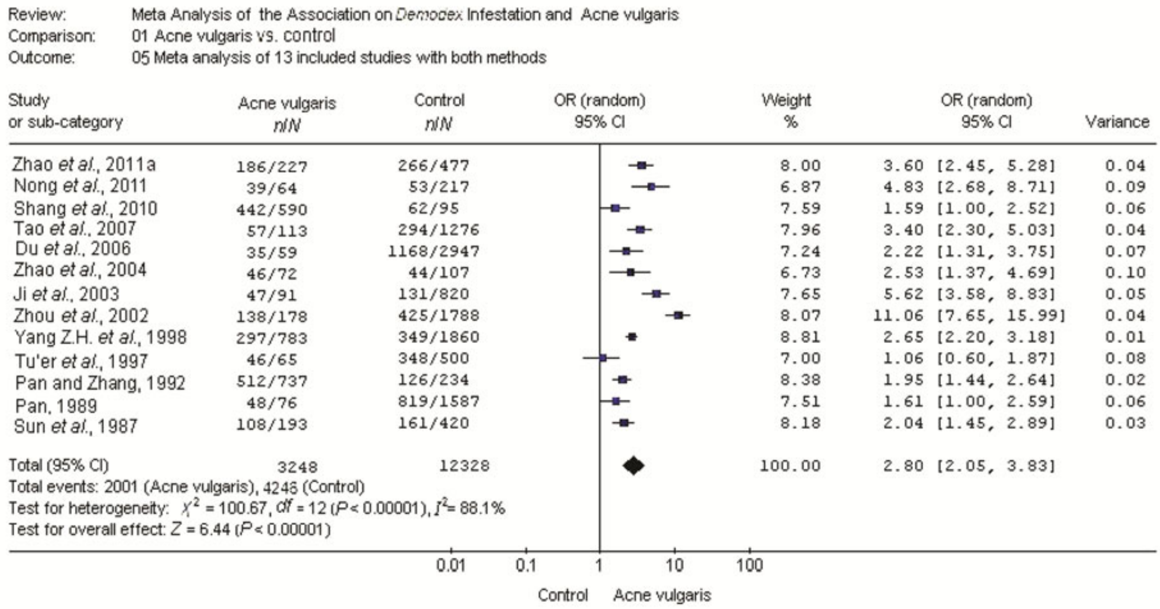

Fig. 5 Forest plot of 13 studies about the association between *Demodex* and acne vulgaris using both the cellophane tape preparation method and skin pressurization method

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 (χ^2=519.09, P<0.01), the results of meta-analysis demonstrated statistical association between *Demodex* 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 18 477 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 (≥5 mites/cm^2) 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±3.9) mites/cm^2, 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±2.6 and 6.8±3.3, respectively, and *Demodex* densities decreased to (1.2±1.0) and (2.2±1.3) mites/cm^2, 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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