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ORIGINAL ARTICLE

Male Infertility

Association of 370-371insACA, 494T>C, and 1423C>T haplotype in ubiquitin-specific protease 26 gene and male infertility: a meta-analysis

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Whether the 370-371insACA, 494T>C, and 1423C>T haplotype in ubiquitin-specific protease 26 (*USP*26) gene is associated with male infertility is controversial. To clarify this issue, we conducted a meta-analysis based on the most recent studies. Eligible studies were screened by using PubMed and Embase. Pooled odd ratio (OR) with 95% confidence interval (CI) was calculated with fixed effect models. Ten studies with 1603 patients and 2505 controls were included. Overall, the results indicated that there was an association between the haplotype and male infertile risk (OR = 1.74, 95% CI: 1.09–2.77). The OR calculated based on the five studies in Asia and three in Europe was 1.96 (95% CI: 1.05–3.67) and 1.54 (95% CI: 0.75–3.16) respectively, however, the OR was 0.86 (95% CI: 0.05–15.29) based on the two investigations in America. In addition, the data from the patients with azoospermia (AZO) showed an increased pooled OR of 2.35 (95% CI: 1.22–4.50). In contrast, the studies with oligoasthenoteratozoospermia (OAT) exhibited that the pooled OR was 0.97 (95% CI: 0.43–2.16). Our analyses indicate that there is an association of alteration in USP26 with male infertility, especially in AZO and Asian population.

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INTRODUCTION

Infertility affects about 10%–15% of couples seeking to conceive, with roughly half of such cases being caused by male factors.[1] The etiology in more than 50% infertile men can not be determined. Recently, great attention has been paid to the genetic causes, including Y chromosome microdeletions, chromosomal aberrations, gene mutation, and gene polymorphisms.[2]

Since men are hemizygous for X-linked genes, meaning that only one single allele is present in an individual, these genes may evolve rapidly when they are exposed to selective pressure.[3,4] Many genes on the X chromosome have been shown to be related to male infertility.[5] Ubiquitin-specific protease 26 (*USP*26), initially identified by Wang *et al.*[6] in 2001, is reported to be one of these genes. *USP*26 is located on Xq26.2, with a single exon. The messenger ribonucleic acid sequence of the USP26 is 2794-bp long with a 52-bp non-coding region at the 5'-terminus, and the protein comprises 913 amino acids (GenBank: NM_031907.1). USP26 belongs to the family of deubiquitinating enzymes (DUBs), which play an important role in the removal of histones and regulation of protein turnover during the spermatogenesis. The expression of USP26 is demonstrated to be predominantly, if not exclusively, in the testes of mouse or human.[6] Because of the importance of DUBs in spermatogenesis, the association of the *USP*26 gene and male infertility has been attracted more attentions.

More than 20 polymorphisms have been reported in the *USP*26 gene and these polymorphisms may form the cluster of alterations.[5] A cluster with three mutations (370-371insACA, 494T>C, and 1423C>T) in the *USP*26 gene is most frequently observed in the male infertile patients. Some investigators demonstrated that this cluster was closely associated with male infertility,[7–10] while others did not find such an association.[11–14] Moreover, the cluster with the three mutations was even not observed in the patients by Zhang *et al.*[15] and Christensen *et al.*[16] Thus, whether the 370-371insACA, 494T>C, and 1423C>T haplotype in *USP*26 gene is associated with male infertility is controversial. Although a previous meta-analysis on this issue was conducted, the analysis included only four articles and did not separately analyze the data derived from different patients with oligoasthenoteratozoospermia (OAT) or azoospermia (AZO).[17] Therefore, we carried out a meta-analysis, including almost all recently published data, to clarify the association between the cluster alteration in USP26 and male infertility, which should be helpful to understand the host factors in the male infertility.

MATERIALS AND METHODS

Search strategy

PubMed and Embase were searched for all relevant English articles published before October 2013. The following terms (alone and in combination) were applied in the search: "male infertility," "semen analysis," "polymorphism," "alternation," "*USP*26," and "ubiquitin-specific protease 26." These keywords reflected that the retrieved articles focused on the relationship between *USP*26 gene

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alternations and male infertility. Two individuals (JDX and JC) independently screened the publications by reviewing titles and abstracts. The references from all original reports and review articles were checked, and the feature of "related citations" in PubMed was also used to further search for possible additional studies.

Inclusion and exclusion criteria

Studies should fulfill all of the following inclusion criteria: (i) published studies or abstracts in English; (ii) case-control association studies on the *USP*26 alternation and male infertility; and (iii) reported measurement of *USP*26 polymorphisms among cases and controls. The articles were not included in the analysis if they met any of (i) incomplete data availability; (ii) duplicated or updated data; or (iii) noninclusion of their own data, such as reviews, comments, editorials, letters, and congress reports.

Data extraction

Two authors (JDX and JC) independently extracted the following information from each study: name of first author, publication year, country of origin, ethnicity of the study population, case (infertility and subgroups) and control definitions, the sample size, and genotypic frequencies. In the end, the accuracy of the data extraction was checked in a second review.

Data analysis

We first aimed to quantify the association between 370-371insACA, 494T>C, and 1423C>T haplotype alternation of *UPS*26 and male infertility, and explore potential sources of heterogeneity. For each alternation reported in the included study, we abstracted data into 2×2 tables and calculated the odds ratio (OR) and the 95% confidence interval (CI), by retrieving the number of cases and controls. The pooled summary OR was estimated by the inverse-variance fixed-effect model (Mantel-Haenszel method) and random-effect model (DerSimonian and Laird method).[18,19] The Chi-squared test and inconsistency index (I-squared [I^2]) were used to estimate the heterogeneity.[20] We evaluated the summary OR with the fixed-effect model, when the P value for heterogeneity was >0.10 and $I^2 < 50\%$, indicating an absence of heterogeneity between studies. In contrast, we applied the random-effect model if $P \leq 0.10$ or $I^2 \geq 50\%$. Subgroup analyses were further performed by various geographic regions (Europe, America, and Asia) and different case types (OAT and AZO). In addition, we conducted the one-way sensitivity analysis to assess the impact of each included study on the overall results and evaluated the stability of the results. Begg's and Egger's tests and inverted funnel plots were utilized to explore the potential publication bias with the linear regression asymmetry test.[21] All statistical analyses were carried out using Stata version 11.0 (Stata Corporation, College Station, TX, USA) and $P < 0.05$ was considered to be significant.

RESULTS

Study characteristics

Initially, a total of 94 articles were identified by searching PubMed and Embase with different combinations of the key terms. After final screening, 10 studies with 1603 cases and 2505 controls were included as they met both the inclusion and exclusion criteria (**Figure 1**). In these 10 studies, the prevalence of the cluster of 370-371insACA, 494T>C, and 1423C>T haplotype changes in the *USP*26 gene varied from 0.0% to 8.6% among all the infertile patients, and from 0.0% to 5.0% among normozoospermic controls. Of the 10 studies, five were conducted in Asian descendants (China, Israel, and Iran), two were carried out in Americans, and the remaining three were performed in Europeans (Belgium and France); six focused on the haplotype in AZO, and four studies also centered upon this issue in OAT. The characteristics of the included studies are described in **Table 1**.

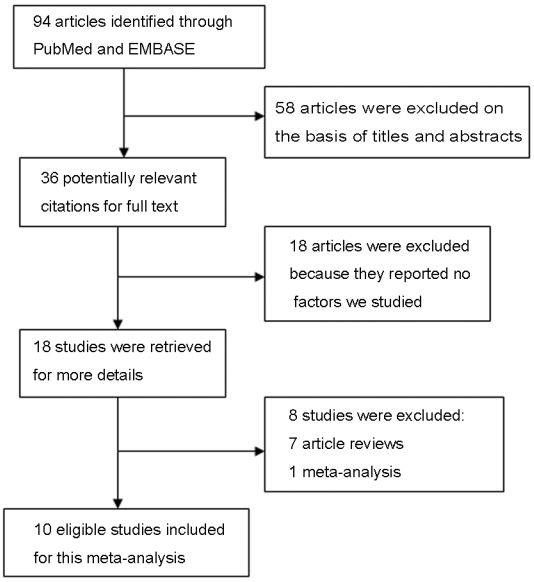

Figure 1: Flow diagram of the studies identified in the meta-analysis.

Meta-analysis

The whole analysis indicated that there was a considerable association between male infertile risk and the 370-371insACA, 494T>C, and 1423C>T haplotype (OR = 1.74, 95% CI: 1.09–2.77, P = 0.019, **Figure 2a**). There was no statistically significant heterogeneity among these studies (P = 0.358). In the stratified analysis by geographic region, the OR calculated based on the five studies in Asia and three in Europe was 1.96 (95% CI: 1.05–3.67, P = 0.035) and 1.54 (95% CI: 0.75–3.16, P = 0.239) respectively, however, the OR was 0.86 (95% CI: 0.05–15.29, P = 0.916) based on the two investigations in America. In addition, the subgroup meta-analysis of the data from the reports on AZO showed a statistically increased pooled OR of 2.35 (95% CI: 1.22–4.50, P = 0.010, **Figure 2b**). In contrast, in the subgroup meta-analysis of the studies with OAT, the pooled OR of 0.97 (95% CI: 0.43–2.16, **Figure 2c**) was statistically not significant (P = 0.933). Heterogeneity was not observed when the subgroup analysis was performed.

Sensitivity analysis and publication bias

To assess the influence of each study on the pooled OR, we conducted the sensitivity analysis through sequentially removing individual studies. The results showed that no individual study significantly affected the overall OR for the association between the cluster of three changes in USP26 and male infertility (**Figure 3**). Publication bias was assessed by a funnel plot, and the Begg's and Egger's tests respectively. As shown in **Figure 4**, the shapes of the funnel plot revealed no obvious asymmetry, suggesting no publication bias, which was further demonstrated by the statistical evidence of Begg's and Egger's test (P = 0.536 and 0.432, respectively).

DISCUSSION

The studies on the relationship between the *USP*26 polymorphisms (370-371insACA, 494T>C, and 1423C>T haplotype) and male infertility risk usually recruited limited number of patients and the findings were controversial. In the present study, we carried

Table 1: Main characteristics of studies included in the meta-analysis

Reference	*Country/ ethnicity*	*Geographic region*	*Genotyping method*	*Cases (%)*			*Controls*	*Patient description*	*Control description*
				AZO	*OAT*	*Total*			
Stouffs *et al.*[7]	Belgium/ mixed	Europe	PCR and Taqman	8/143 (5.4)	NA	8/143 (7.2)	0/152 (0.0)	Sertoli cell-only syndrome, normal karyotype, no Yq microdeletions	Normal sperm parameters
Paduch *et al.*[8]	USA/ unknown	Americas	PCR and HPLC	4/188	NA	4/188 (2.1)	0/17 (0.0)	Non-obstructive azoospermia with no chromosomal aberrations or Yq microdeletions	Fertile men
Ravel *et al.*[11]	France/ mixed	Europe	PCR and Taqman	4/40 (10.0)	1/59 (1.7)	5/99 (5.1)	75/1334 (4.0)	Azoospermia or oligozoospermia with no Y or gr/gr microdeletions	Fertility or sperm count known
Stouffs *et al.*[12]	Belgium/ Caucasian	Europe	PCR and Taqman	NA	0/146 (0.0)	0/146 (0.0)	1/202 (0.5)	Without abnormal karyotype or Yq microdeletions	Normal spermat-ogenesis, sperm parameters, or proven fertility
Zhang *et al.*[15]	China/Han nationality	Asia	PCR and SSCP	NA	NA	0/44 (0.0)	0/56 (0.0)	No immue, infection and biochemical abnormality	Normal sperm parameters
Lee *et al.*[9]	China/Han nationality	Asia	PCR and Taqman	NA	NA	6/200 (3.0)	0/200 (0.0)	Without any identifiable cause of male infertility	Fathered at least two children with 5 years without any assisted reproductive technologies
Christensen *et al.*[16]	USA/ unknown	Americas	PCR	0/48 (0.0)	0/48 (0.0)	0/96 (0.0)	0/96 (0.0)	Azoospermic and oligozoospermic	Unknown semen analysis parameters but known paternity
Ribarski *et al.*[13]	Israel/mixed	Asia	PCR and Taqman	NA	NA	9/300 (3.0)	6/287 (2.1)	Azoospermic, oligozoospermic and infertile with unknown semen parameters	Proved fertile
Shi *et al.*[14]	China/Han nationality	Asia	PCR	5/60 (8.3)	14/161 (8.7)	19/221 (8.6)	5/101 (5.0)	46, XY karyotype, no AZF microdeletion, no identifiable causes of male infertility	Fertile men with normozoospermia
Asadpor *et al.*[10]	Iran/ unknown	Asia	PCR, SSCP, and Taqman	4/166 (2.4)	NA	4/166 (2.4)	1/60 (1.7)	No history with cystic fibrosis, Klinefelter syndrome, varicocele, chemotherapy, AZF genes micro deletions, etc	Normal semen, at least one child within 3 years without assisted reproductive technologies and no history of miscarriages

All values represent number of men: each numerator represents the number of men with the 370-371insACA, 494T>C, and 1423C>T haplotype cluster change in USP26; each denominator represents the total number of men with the characteristic as indicated in the column header. AZO: azoospermia; HPLC: high performance liquid chromatography; OAT: oligoasthenoteratozoospermia; SSCP: single strand conformation polymorphism; NA: not available; AZF: azoospermia factor; USP26: ubiquitin-specific protease 26; PCR: polymerase chain reaction

out a meta-analysis of 10 studies involving 1603 patients and 2505 controls. The overall results support the hypothesis that the *USP*26 polymorphisms are associated with male idiopathic infertility. More specifically, the haplotype may increase the infertility risk in AZO patients and Asian male population.

Spermatogenesis is a developmental process, in which the germ stem cells go through proliferation, meiosis and spermiogenesis to form spermatozoa. DUBs negatively regulate protein ubiquitination, which is involved in the control of meiosis and reorganization of chromatin structure during spermatogenesis.[22,23] Based on the recent studies,[24,25] DUBs are functionally divided into five main families: ubiquitin C-terminal hydrolases, ubiquitin-specific processing proteases (USPs or UBPs), ovarian tumor-domain ubiquitinaldehyde-binding proteins, Jab1/Pad1/MPN-domain-containing metalloenzymes, and Atain-3/Josephin.[25] USP26 belongs to the USPs or UBPs family, and may play an important role in the regulation of protein turnover.[7] Recently, Dirac and Bernards have found that USP26 encodes a nuclear protein that binds to the androgen receptor (AR) and modulates AR ubiquitination, consequently influencing AR activity and stability.[26] The AR signaling pathway is essential for maintenance of spermatogenesis. The 370-371insACA, 494T>C, and 1423C>T cluster haplotype results in T123-124ins, L165S, and H475Y mutations, respectively.[7] In a study by Ribarski *et al.*[13] they found that the amino acids at positions 122 and 475 were relatively easily evolutionary, whereas that at position 165 was less evolutionary. They also identified three putative phosphorylation motifs: two started at position 123 and the third was initiated at 577. Mutation 370-371insACA, resulting in insertion of an additional T at position 123, moved the phosphorylation motif one position ahead, while the mutation 1423C>T (H475Y) disturbed the 3D structure of USP26 protein.[13]

Azoospermia is categorized as obstructive and nonobstructive. In agreement with the findings that men with nonobstructive AZO commonly have genetic abnormalities,[27] our results indicated that the alternation of USP26 is associated with AZO. Thus, USP26 may play an important role in spermatogenesis. On the other hand, OAT is classified as isolated mild (astheno and/or teratospermia), moderate, and severe.[28] The etiology of OAT is associated more closely with the environmental factors, such as age, noninflammatory functional alternations in posttesticular organs, infectious agents, environmental pollutants, and "subtle" hormonal abnormalities.[29] This is in accordance with the findings that USP26 haplotype is not associated with OAT in the present study.

Interestingly, in the stratified analysis by geographic regions, we found this association was significant only in Asian population. This is in line with Ravel *et al.*,[11] indicating that the association of USP26 haplotype and male infertility is dependent upon the ethnic origins. Some studies also have suggested associations between gene polymorphisms and male infertility may vary in the different ethnics,

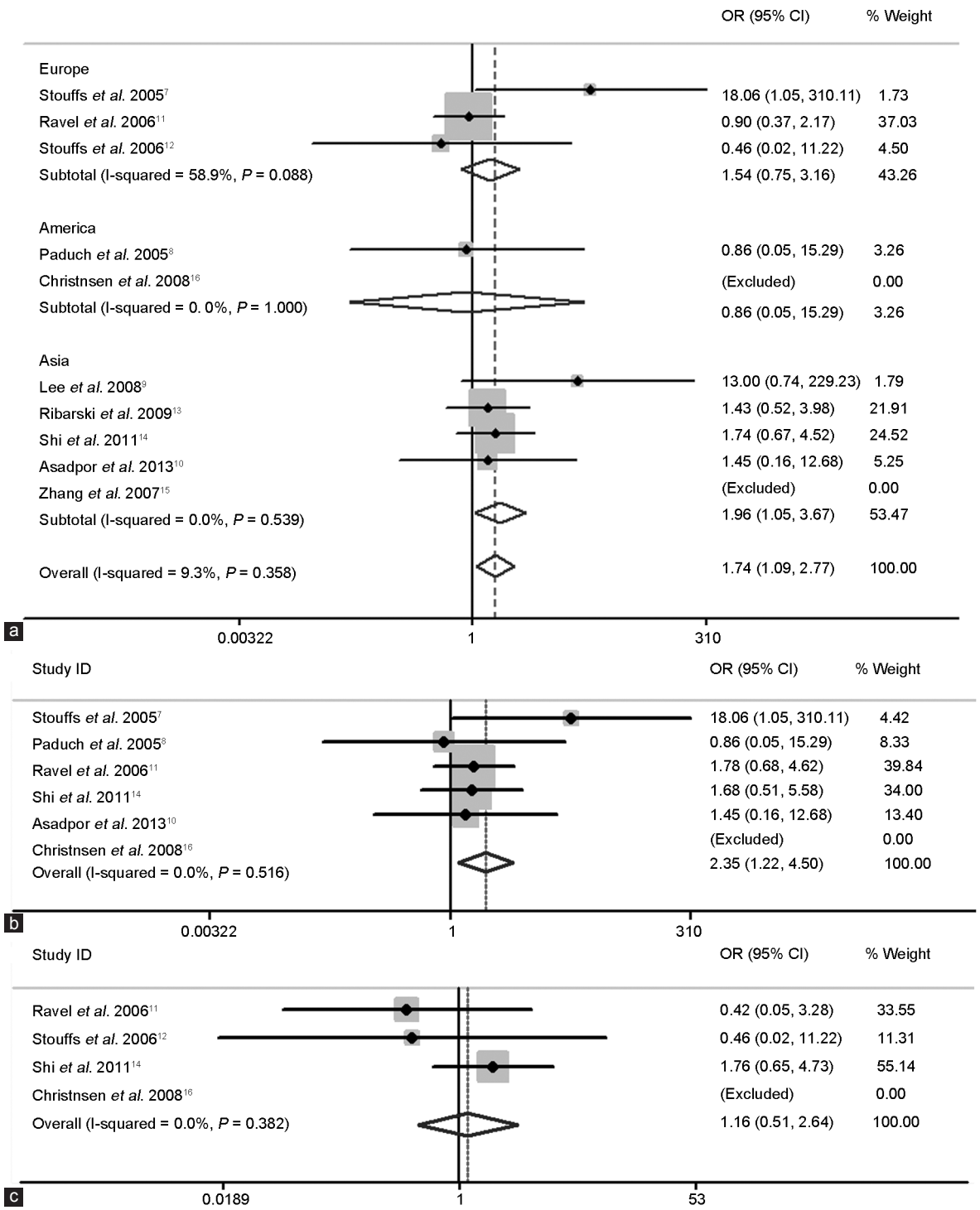

Figure 2: Forest plot of the studies assessing the association between the 370-371insACA, 494T>C, and 1423C>T haplotype in USP26 and male infertility (**a**), azoospermia (**b**), and oligoasthenoteratozoospermia (**c**). Horizontal lines indicate 95% confidence interval (CI); diamonds indicate summary relative risk estimate with its corresponding 95% CI.

such as deleted azoospermia-like and polymerase gamma genes.[30] Hence, the genetic background combined with the environmental factors may lead to spermatogenetic impairment.[31]

A main limitation of the present study is that some studies with small sample size were included, which could increase the likelihood of type I and type II errors. However, totally 10 studies with 1603 cases and 2505 controls were analyzed, which may minimize the statistical bias. In addition, the overall results were analyzed with unadjusted estimates. However, other potential factors such as age, body mass index, and smoke or alcohol habits were not available in the included articles.

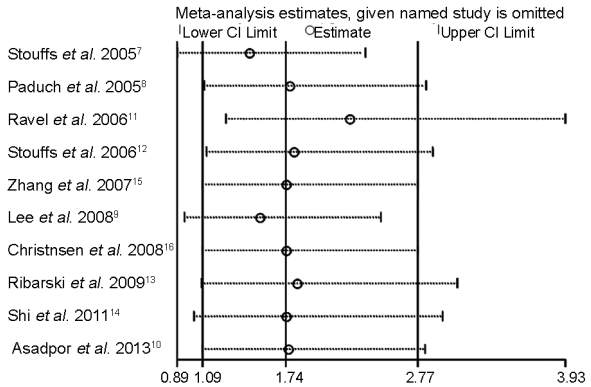

Figure 3: Sensitivity analysis diagram for each study used to assess the relative risk estimates for the 370-371insACA, 494T>C, and 1423C>T haplotype in USP26 and male infertility in all the included studies.

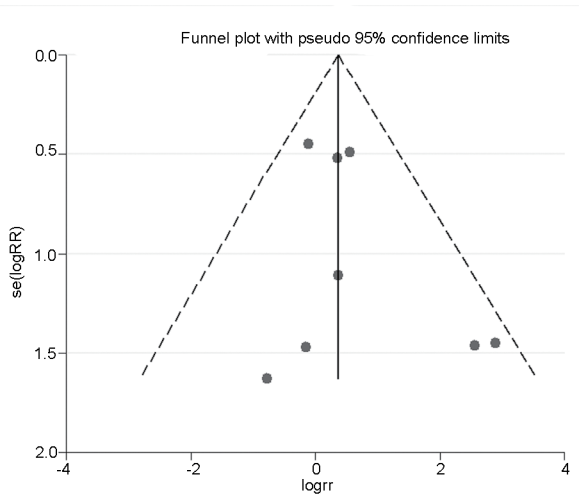

Figure 4: Publication bias in all the studies. Both visualization of funnel plot and Begg's test ($P = 0.536$), or Egger's test ($P = 0.432$) indicated no publication bias in the studies included in the meta-analysis.

CONCLUSIONS

Our meta-analysis suggests that the 370-371insACA, 494T>C, and 1423C>T haplotype cluster in *USP26* gene is associated with male infertility, especially in AZO and Asian population. Well-designed studies with large sample size are warranted to validate the association.

AUTHOR CONTRIBUTIONS

JDX and YTD conceived and designed the study. JDX, JC, YFH, HC, WY, and YC collected the data. JDX, JC, and YFH performed the statistical analysis. JDX, JC, and YTD drafted and revised the manuscript. All authors read and approved the final version.

COMPETING INTERESTS

The authors declare no competing interests.

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