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 (USP26) 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 odds 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 infertility risk (OR = 1.74, 95% CI: 0.95–2.77). 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% of infertile men cannot 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.³⁴ Many genes on the X chromosome have been shown to be related to male infertility.⁵ Ubiquitin-specific protease 26 (USP26), initially identified by Wang et al.⁶ in 2001, is reported to be one of these genes. USP26 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.⁷ Because of the importance of DUBs in spermatogenesis, the association of the USP26 gene and male infertility has been attracted more attention.

More than 20 polymorphisms have been reported in the USP26 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 USP26 gene is most frequently observed in the male infertile patients. Some investigators demonstrated that this cluster was closely associated with male infertility,²⁻⁵⁻¹⁰ 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 USP26 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," "USP26," and "ubiquitin-specific protease 26." These keywords reflected that the retrieved articles focused on the relationship between USP26 gene...
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 USP26 alternation and male infertility; and (iii) reported measurement of USP26 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 USP26 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). The Chi-squared test and inconsistency index (I²) were used to estimate the heterogeneity. We evaluated the summary OR with the fixed-effect model, when the P value for heterogeneity was >0.10 and F < 50%, indicating an absence of heterogeneity between studies. In contrast, we applied the random-effect model if P ≤ 0.10 or F ≥ 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. 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 in the meta-analysis. 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 USP26 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
out a meta-analysis of 10 studies involving 1603 patients and 2505 controls. The overall results support the hypothesis that the USP26 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 spermatooza. DUBs negatively regulate protein ubiquitination, which is involved in the control of meiosis and reorganization of chromatid structure during spermatogenesis. Based on the recent studies, 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. USP26 belongs to the USPs or UBPs family, and may play an important role in the regulation of protein turnover. Recently, Dirac and Bernards have found that USP26 encodes a nuclear ubiquitin-specific protease 26 (USP26), and that the mutation 1423C>T (H475Y) disturbed the 3D structure of USP26 protein. While the mutation 370-371insACA, resulting in insertion of an additional T at position 123, moved the phosphorylation motif one position ahead, mutation 370-371insACA, 494T>C, and 1423C>T cluster haplotype results in less evolutionary. They also identified three putative phosphorylation motifs: two started at position 123 and the third was initiated at 577. They also identified three putative phosphorylation motifs: two started at position 123 and the third was initiated at 577.

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

| Reference        | Country/Ethnicity | Geographic Region | Genotyping Method | Cases (%) | Controls | Patient Description | Control Description |
|------------------|-------------------|-------------------|-------------------|-----------|----------|---------------------|---------------------|
| 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 |
| 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 |
| 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) | 0/166 (2.4) | 0/1030 (0.5) | Azoospermia or oligozoospermia with no Y or gr/gr microdeletions |
| Stouffs et al.12 | Belgium/Caucasian | Europe            | PCR and Taqman    | 0/146 (0.0) 0/146 (0.0) 1/202 (0.5) | 0/146 (0.0) | 0/200 (0.0) | Without abnormal karyotype or Yq microdeletions |
| Zhang et al.15   | China/Han nationality | Asia | PCR and SSCP | 0/44 (0.0) | 0/44 (0.0) | 0/56 (0.0) | Normal sperm-ogenesis, sperm parameters, or proven fertility |
| Lee et al.3      | China/Han nationality | Asia | PCR and Taqman | 0/6200 (3.0) | 0/6200 (3.0) | 0/200 (0.0) | Normal sperm parameters |
| Christensen et al.16 | USA/unknown | Americas | PCR | 0/048 (0.0) 0/048 (0.0) 0/96 (0.0) 0/96 (0.0) | 0/6200 (3.0) | 0/6200 (3.0) | Azoospermic and oligozoospermic |
| Ribarski et al.13 | Israel/mixed | Asia | PCR and Taqman | 0/9300 (3.0) | 0/9300 (3.0) | 0/287 (2.1) | Normal semen analysis parameters but known paternity |
| Shi et al.14     | China/Han nationality | Asia | PCR | 0/14160 (8.3) 19/221 (8.6) 5/101 (5.0) | 0/14160 (8.3) | 0/14160 (8.3) | Normal semen, at least one child within 3 years without assisted reproductive technologies and no history of miscarriages |
| Asadpor et al.10 | Iran/unknown      | Asia | PCR, SSCP, and Taqman | 0/166 (2.4) | 0/166 (2.4) | 1/60 (1.7) | Fertile men with normozoospermia |

All values represent number of men; each numerator represents the 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.

This is in accordance with the findings that men with nonobstructive AZO commonly have genetic abnormalities, 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. The etiology of OAT is associated more closely with environmental factors, such as age, noninflammatory functional and severe. The etiology of OAT is associated more closely with environmental factors, such as age, noninflammatory functional and "subtle" hormonal abnormalities. 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. 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.
such as deleted azoospermia-like and polymerase gamma genes. Hence, the genetic background combined with the environmental factors may lead to spermatogenetic impairment.

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 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.
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