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ORIGINAL ARTICLE

Male Infertility

Association of polymorphisms of A260G and A386G in *DAZL* gene with male infertility: a meta-analysis and systemic review

Ping Chen, Xiao Wang, Chang Xu, He Xiao, Wen-Hao Zhang, Xing-Huan Wang, Xin-Hua Zhang

To investigate the association of single nucleotide polymorphism 260 and 386 (SNP260 and SNP386) gene with male infertility, an electronic search was performed to identify case-control studies evaluating the relationship of SNP260 or SNP386 of deleted in azoospermia-like (*DAZL*) and male infertility. Review Manager 5 was used to process the meta-analysis and other statistical analysis. A total of 139 records were retrieved, of which 13 case-control studies with total 2715 patients and 1835 normozoospermic men were included. SNP260 was found not to play a functional role in male oligo/azoospermia either for Caucasians or for Asians. But for SNP386, models of allele (A/G), dominant (AA/AG + GG), co-dominant (AA/AG) and super-dominant (AA + GG/AG) had a strong correlation to spermatogenic failure with related odds ratio being 0.15 (95% confidence interval [95% CI] 0.07 to 0.34, $P < 0.00001$), 0.16 (95% CI 0.07 to 0.35, $P < 0.00001$), 0.15 (95% CI 0.06 to 0.33, $P < 0.00001$) and 0.15 (95% CI 0.06 to 0.33, $P < 0.00001$), respectively. Moreover, this correlation was only found in the Chinese Han population (decreasing around 85% risk of oligo/azoospermia infertility) and not found in India, Japan, and Caucasian countries. Our analysis demonstrated that SNP260 of *DAZL* did not contribute to oligo/azoospermia while SNP386 was correlated to male infertility. However, this correlation was only found in China with a country-specific and ethnicity-specific manner.

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INTRODUCTION

The prevalence of infertility may be as high as 15% with about 50% of cases attributed to male factors. Male infertility is commonly believed to result from an exogenous insult after birth or *in utero*, such as an exposure to gonadotoxins, trauma, or infection, but there is no effective treatment for patients with nonobstructive azoospermia, in which there is an absence of mature sperm in the testes. This suggests the exogenous insult after birth or *in utero* may not play a role for nonobstructive azoospermia patients.[1] But from an evidence-based medicine perspective, rigorous studies demonstrating direct cause and effect relationship in human infertility are frequently lacking. Changes in sperm motility parameters are the predominant factor in most cases of male infertility, however, in 30%–45%, the cause of the abnormal sperm parameters is not identified (idiopathic male infertility).[2] In recent years, there has been remarkable progress in understanding the regulation of spermatogenesis and the causes of male infertility, largely as a result of the molecular and biochemical insights. It is estimated that in about 30% of cases, male infertility is due to a genetic disorder such as aneuploidy, structural chromosomal abnormalities, DNA damage, and gene mutations including a variety of newly discovered genes.[3] The most frequent causes are rare variants causing the spermatogenic dysfunction.

The incidence of chromosomal abnormalities is 5.8% for infertile men.[4] Of these, sex chromosome abnormalities account for 4.2% and autosomal abnormalities for 1.5%. Among the azoospermia/severe oligozoospermia patients, microdeletions of the Y chromosome, including *deleted in azoospermia* (*DAZ*) gene family, are the major, well-characterized genetic causes. *DAZ-like* (*DAZL*), an autosomal homolog of *DAZ*, localized on chromosome 3 and expressed in germ cells, is essential for germ cell lineage development in several species.[5,6] Mutation analysis of *DAZL* in infertile men identified the first two nonsynonymous single nucleotide polymorphism (SNP) at nucleotide position 260 (exon 2) and 386 (exon 3), resulting in the amino acid exchange T12A and T54A, respectively.[7] In recent years, a number of studies investigated the possible association of azoospermia or oligozoospermia with A260G or A386G sites polymorphism but controversies exist. Tüttelmann *et al.*[8] summarized all polymorphisms and male infertility that have been investigated in single case-control studies in their meta-analysis in 2007 and revealed significant associations between polymorphism and spermatogenic failure only for *AZF gr/gr* deletions and *MTHFR* 677C → T but not for *POLG*, *DAZL*, *USP26*, or *FSHR*. However, for *DAZL* polymorphism, just A260G gene loci were analyzed with only six included studies in their review. Thus, no complete meta-analysis has been conducted to verify this correlation. In this review, we apply the methods of evidence-based medicine to evaluate and analyze the documented studies of *DAZL* polymorphism and male infertility so as to provide a more systematic and comprehensive assessment of their associations.

Department of Urology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China.
Correspondence: Dr. XH Zhang (zxhmd2000@yahoo.com)
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MATERIALS AND METHODS

Inclusion criteria

Case-control studies investigated the association of SNP260 or SNP386 of *DAZL* with male infertility should provide sufficient data that could be extracted and used to calculate odds ratios (ORs) and 95% confidence intervals (95% CI). Patients were diagnosed as male infertility with sperm count $<20 \times 10^6$ ml^{-1} or no sperm in at least two seminal fluid examinations after 3/4 days of sexual abstinence, which was accordant with 2012 European Association of Urology[9] or 2010 WHO Laboratory Manual for the Examination and Processing of Human Semen.[10] There was no limitation to the methods of assays for detecting SNP of *DAZL*.

Exclusion criteria

Repeat publications and grey literature that were unpublished and reported superficially studies, such as in the form of an abstract and the studies in which sample size <10 or main data could not be obtained would be excluded.

Literature search strategy

We performed an electronic search of PubMed (1966 to September 2013), ScienceDirect Online (1995 to September 2013), Wiley Online Library (2010 to September 2013), China National Knowledge Infrastructure (CNKI) (1999 to September 2013), VIP Database (2000 to September 2013) and Chinese Dissertation Database (CDDB) (1998 to September 2013) for case-control studies evaluating the relationship of SNP260 or SNP386 of *DAZL* and male idiopathic oligozoospermia (sperm number $<20 \times 10^6$ spermatozoa per ml) or azoospermia. The search keywords were used with different combinations with both medical subject headings terms and text words: "male infertility" or "male sterility" or "male infecundity" or "male sterile" or "male dysgenesis" or "azoospermia" or "oligozoospermia" or "azoospermic" or "oligozoospermic" or "OAT" plus "*DAZL*" or "*deleted in azoospermia-like*" or "*spgyla*" or "*Daz-like* autosomal" or "SNP260" or "SNP386" or "T12A" or "T54A" or "SNP." Publication date was not restricted in our search. Reference lists of the included studies and supplemental materials were checked manually to further identify related studies. Meanwhile, published genome-wide association studies (GWASs) about male infertility were also examined.

Selection of studies

Two reviewers (CX and HX) independently screened the title, abstract, and keywords of each article retrieved. Full-text papers were screened for further assessment if the information given suggested that the study fulfilled the inclusion criteria and did not meet the exclusion criteria. Discrepancies were settled by discussion and consensus with all the authors.

Data extraction

The following information was independently extracted from the identified studies by three reviewers (CX, HX and WHZ) using a standard form with first author's surname, year of publication, country, ethnicity, allele frequencies, and genotype distribution in cases and controls, method of genotype test and Hardy–Weinberg equilibrium (HWE) test. Ethnicities were stratified as Asians, Caucasians, and others. The authors of original studies were consulted for missing information where necessary. Discrepancies were resolved by open discussion.

HWE test

HWE test was performed, and the HWE significance of the control groups was calculated with StataSE 12.0 (StataCorp LP, College Station, TX, USA) when the original information was not provided.

Data synthesis and analysis

The significance for five genetic models (allele, dominant, recessive, co-dominant, and super-dominant genetic models) was evaluated for each study separately. All the associations were indicated as ORs with the corresponding 95% CI. Based on the individual ORs, a pooled OR was estimated. Subgroup analysis was also performed by stratifying country and ethnicity. Fixed-effects model[11] or the random-effects model[12] of meta-analysis was chosen according to the results of heterogeneity tests among individual studies by Review Manager 5 (The Cochrane Library). The significance of the pooled OR was determined using the Z test and $P < 0.05$ was considered statistically significant.

Heterogeneity assumption was assessed by Cochran's Q statistic[13] and I^2 statistic. The heterogeneity was considered statistically significant if $P < 0.10$. The random-effects model (if $P < 0.10$ and $I^2 > 50\%$) or the fixed-effects model (if $P \geq 0.10$ and $I^2 < 50\%$) was used to pool the ORs. Egger's test was used to evaluate the publication bias,which was considered when $P < 0.05$.

RESULTS

Characteristics of included studies

Using the database search strategy, a total of 139 records were retrieved from PubMed, Science Direct, Wiley Online Library, CNKI, VIP and CDDB, of which 13 case-control studies finally met full inclusion criteria for this review.[7,14–25] No dataset of genotype frequencies of SNP was acquired from GWASs of male infertility.[26,27] **Supplementary Figure 1** depicts the flowchart of the search process. **Supplementary Table 1** describes the characteristics of the included trials. **Supplementary Tables 2** and **3** describe the distribution of genotypes and allele frequencies of SNP260 and SNP386 in infertile men and control group, respectively.

Meta-analysis of single nucleotide polymorphism 260

A total of ten studies were included for the analysis of SNP260. **Figure 1** showed the meta-analysis for allele model (A/G), dominant model (AA/AG + GG), recessive model (AA + AG/GG), co-dominant model (AA/AG), co-dominant model (AA/GG) and super-dominant model (AA + GG/AG), respectively. I^2 standing for the heterogeneity among studies for all models was 0%; thus, fixed-effects models were applied. Related ORs of the six models were 0.88 (95% CI 0.73 to 1.07, $P = 0.21$), 0.85 (95% CI 0.69 to 1.05, $P = 0.12$), 0.70 (95% CI 0.31 to 1.58, $P = 0.39$), 0.83 (95% CI 0.67 to 1.02, $P = 0.08$), 1.39 (95% CI 0.61 to 3.17, $P = 0.43$) and 0.82 (95% CI 0.66 to 1.02, $P = 0.08$), respectively, which indicated that SNP260 was not significantly associated with male infertility for all models. Sensitivity analysis was performed by eliminating each of the included studies, and the statistical significance was not changed, suggesting that the results above were reliable. Egger's test revealed that there was no any obvious evidence of publication bias ($P = 0.737$).

Table 1 shows the summary of the subgroup analysis stratified by different ethnicity. No significant difference was detected between each subgroup, either.

Meta-analysis of single nucleotide polymorphism 386

Single nucleotide polymorphism 386 of *DAZL* was not found in populations from Germany, Italy, Japan, Northern China, and Western India. It was only detected in populations from Western China, Taiwan of China, Northern, and Eastern India. **Figure 2** showed the meta-analysis for the allele model (A/G), dominant model (AA/AG + GG), recessive model (AA + AG/GG), co-dominant model (AA/AG), co-dominant model (AA/GG), and super-dominant model (AA + GG/AG), in

which I^2 standing for the heterogeneity among the studies was 19%, 28%, 0%, 38%, 0%, and 38%, respectively. Thus fixed-effects models were applied. Related OR was 0.15 (95% CI 0.07 to 0.34, $P < 0.00001$), 0.16 (95% CI 0.07 to 0.35, $P < 0.00001$), 2.11 (95% CI 0.24 to 18.67, $P = 0.50$), 0.15 (95% CI 0.06 to 0.33, $P < 0.00001$), 0.45 (95% CI 0.05 to 4.04, $P = 0.48$) and 0.15 (95% CI 0.06 to 0.33, $P < 0.00001$), indicating that model of A/G, AA/AG + GG, AA + GG/AG AA/AG of SNP386 was significantly associated with spermatogenic failure, while for model AA + AG/GG and AA/GG, the difference was not significant. Sensitivity analysis was performed by eliminating each of the included studies, and the statistical significance was not changed, suggesting that the results above were reliable. Egger's test revealed that there was obvious evidence of publication bias ($P = 0.009$).

Table 1 shows subgroup analysis stratified by different countries. For allele model (A/G), dominant model (AA/AG + GG), super-dominant model (AA + GG/AG) and co-dominant model (AA/AG) model, we detected significant association of SNP386 with male infertility. But the significant difference was kept consistent only in China. In co-dominant model (AA/GG) and recessive model (AA + AG/GG), the genotype of GG was not detected in India and meta-analysis of this subgroup was not applicable.

DISCUSSION

This meta-analysis and systemic review investigated the association of *DAZL* polymorphisms and male infertility. Our novel data demonstrated that SNP260 of *DAZL* did not contribute to oligozoospermia or azoospermia, while SNP386 of *DAZL* was correlated to male infertility only in the Chinese Han.

Polymorphisms or genetic variants in genes involved in spermatogenesis are considered potential risk factors which may contribute to the severity of spermatogenic failure. Several polymorphic variants have been described in association with oligo/

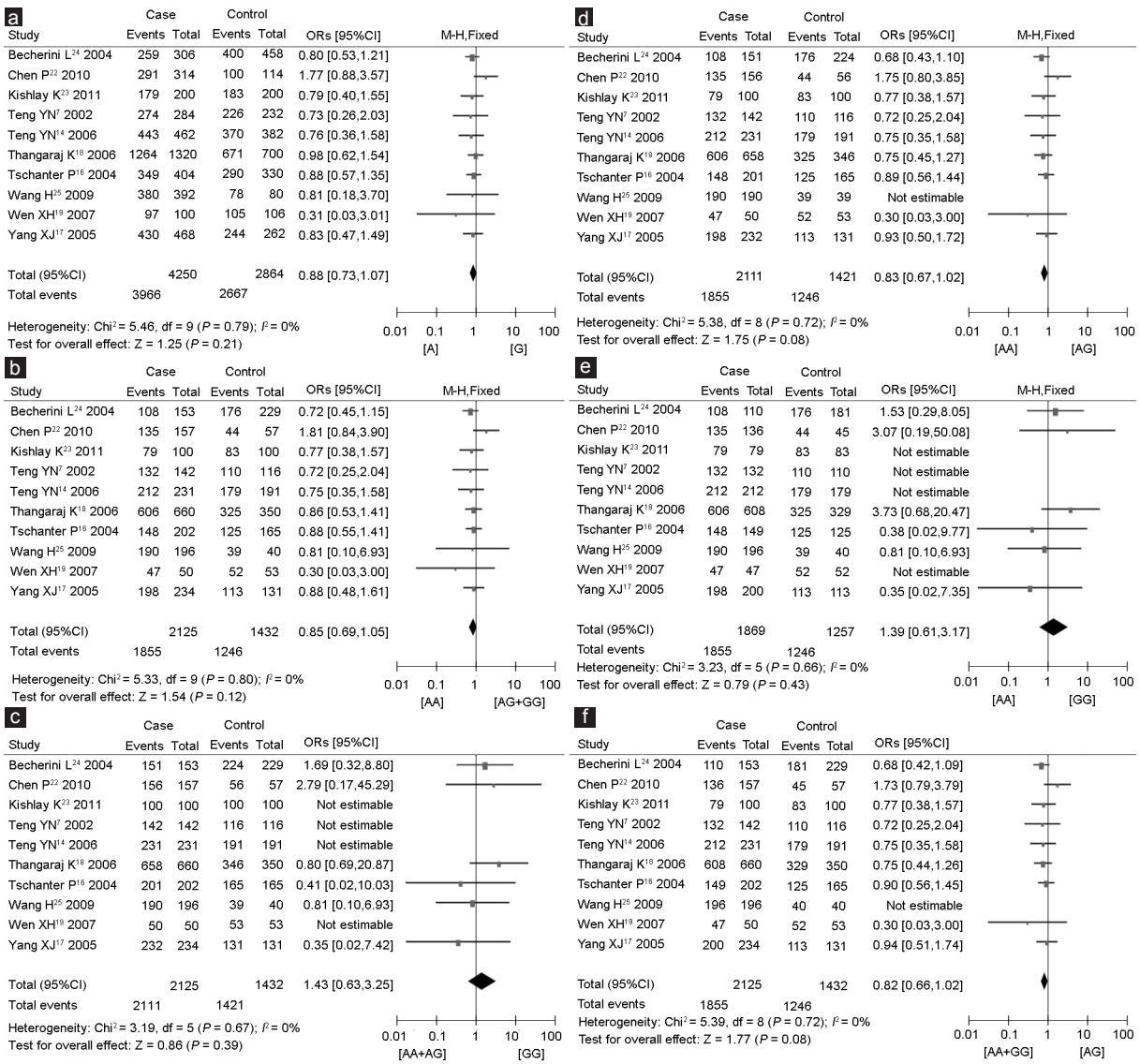

Figure 1: The association of single nucleotide polymorphism 260 with male infertility. A forest plot in (**a**) allele model (A/G), (**b**) dominant model (AA/AG + GG), (**c**) recessive model (AA + AG/GG), (**d**) co-dominant model (AA/AG), (**e**) co-dominant model (AA/GG), (**f**) super-dominant model (AA + GG/AG). The association was indicated as odds ratio (OR) estimate with the corresponding 95% confidence interval (CI). The OR estimate of each study is marked with a solid black square. The size of the square represents the weight that the corresponding study exerts in the meta-analysis. The CIs of pooled estimates are displayed as a horizontal line through the diamond. OR <1 indicates increased risk of male infertility. A: wild type gene, G: mutant gene.

azoospermia,[28–35] among which two *DAZL* polymorphisms (SNP260 and SNP386) were the first description of SNPs of autosomal genes influencing human spermatogenesis. Analysis of the distribution and frequency of these SNPs among fertile and infertile men has demonstrated a strong association of the heterozygous genotype for SNP386 with spermatogenic failure for Asians while it was not detected in Caucasians. But no such association could be found for SNP260.

In order to provide a complete assessment, we performed the present meta-analysis of 13 independent case-control studies with total number of 2715 patients and 1835 normozoospermic men, of which there were 2500 oligo/azoospermic men. We found that SNP260 of *DAZL* had no correlation with male oligozoospermia or azoospermia which was consistent with Tüttelmann's meta-analysis[8] including 6 studies with 1600 patients and 1100 controls of different ethnic origin. In this previous review,[8] the authors did not perform meta-analysis on the relationship between SNP386 and male infertility. Instead, they did a literature review and drew a conclusion that SNP386 was never found in non-Chinese populations. However, SNP386 was later detected in India though there was no association with male infertility.

We further conducted a subgroup analysis by ethnicity and country in the current meta-analysis. In the stratified analysis according to ethnicity, we found that SNP260 had no correlation with male oligozoospermia or azoospermia either in Caucasian or Asian populations. For SNP386, it was only detected in Asians and not in Caucasians. We also further performed the stratified analysis by country and demonstrated that models of SNP386 of allele (A/G), dominant (AA/AG + GG), co-dominant (AA/AG), and super-dominant (AA + GG/AG) had a strong association with spermatogenic failure in China. Although these variants were detected in northern and eastern India, the difference did not reach statistical significance. Therefore, functional *DAZL* A/G polymorphism may play a penetrance role in male oligo/azoospermia susceptibility in ethnicity-specific and country-specific manners.

Discrepancies between results of association studies are a rather frequent phenomenon and may be related to many different factors.[36]

Table 1: Subgroup analysis stratified by different population and countries

Gene site	*Genetic model*	*Population*	*P Q test*	*I^2 (%)*	*P Z test*	*OR (95% CI)*
SNP260	Allele (A/G)	Overall	0.79	0	0.21	0.88 (0.73–1.07)
		Caucasian	0.77	0	0.24	0.83 (0.62–1.13)
		Asian	0.65	0	0.52	0.92 (0.71–1.19)
	Dominant (AA/AG+GG)	Overall	0.8	0	0.12	0.85 (0.69–1.05)
		Caucasian	0.57	0	0.18	0.80 (0.57–1.11)
		Asian	0.69	0	0.37	0.88 (0.67–1.16)
	Recessive (GG/AA+AG)	Overall	0.67	0	0.86	1.43 (0.63–3.25)
		Caucasian	0.44	0	0.28	1.22 (0.31–4.87)
		Asian	0.48	0	0.88	1.57 (0.58–4.29)
	Co-dominant (AA/AG)	Overall	0.72	0	0.08	0.83 (0.67–1.02)
		Caucasian	0.44	0	0.16	0.78 (0.56–1.10)
		Asian	0.6	0	0.27	0.85 (0.65–1.13)
	Co-dominant (AA/GG)	Overall	0.66	0	0.43	1.39 (0.60–3.17)
		Caucasian	0.46	0	0.87	1.12 (0.28–4.52)
		Asian	0.47	0	0.38	1.57 (0.58–4.30)
	Super-dominant (AA+GG/AG)	Overall	0.72	0	0.08	0.82 (0.66–1.02)
		Caucasian	0.41	0	0.15	0.78 (0.56–1.09)
		Asian	0.61	0	0.26	0.85 (0.65–1.13)
SNP386	Allele (A/G)	Overall	0.29	19	<0.01	0.15 (0.07–0.34)
		India	0.4	0	0.79	0.80 (0.16–4.09)
		China	0.79	0	<0.01	0.11 (0.04–0.28)
	Dominant (AA/AG+GG)	Overall	0.23	28	<0.01	0.16 (0.07–0.35)
		India	0.4	0	0.79	0.80 (0.16–4.09)
		China	0.53	0	<0.01	0.11 (0.04–0.28)
	Recessive (GG/AA+AG)	Overall	0.89	0	0.5	2.11 (0.24–18.67)
		India	-	-	-	-
		China	0.89	0	0.5	2.11 (0.24–18.67)
	Co-dominant (AA/AG)	Overall	0.19	38	<0.01	0.15 (0.06–0.33)
		India	0.4	0	0.79	0.80 (0.16–4.09)
		China	0.88	0	<0.01	0.09 (0.03–0.26)
	Co-dominant (AA/GG)	Overall	0.86	0	0.48	0.45 (0.05–4.04)
		India	-	-	-	-
		China	0.86	0	0.48	0.45 (0.05–4.04)
	Super-dominant (AA+GG/AG)	Overall	0.19	38	<0.01	0.15 (0.06–0.33)
		India	0.4	0	0.79	0.80 (0.16–4.09)
		China	0.89	0	<0.01	0.09 (0.03–0.26)

OR: odds ratio; CI: confidence interval; SNP: single nucleotide polymorphisms

An important feature of these studies is that the discrepancy between Caucasian analysis and the Chinese studies is not related to sampling biases (small sample size, inadequate control group or population substructuring), but it is related to the complete absence of the "at risk" SNP in Caucasians, indicating that in Western countries, this polymorphism is probably absent or rare. This remarkable difference is paradigmatic as it shows how ethnic background is important for polymorphisms involved in spermatogenesis, thereby underscoring that different genetic risk factors may be present in different populations. A similar phenomenon is observed for *cystic fibrosis transmembrane conductance regulator* (*CFTR*) gene mutations causing the azoospermia because of congenital bilateral absence of vas deferens. The frequency of a particular *CFTR* mutation is also influenced by the ethnic composition of the population analyzed.[37] Our study, therefore, contributes to a better definition of clinically relevant tests, specifically based on the ethnic origin of the infertile patients.

The low heterogeneity in our analysis contributes to highly reliable results through meta-analysis. There is no publication bias in the studies about *DAZL* SNP260. But there exists publication bias about SNP386 of *DAZL*. The sources of bias might be from the small sample sizes or incorporated literatures of low quality. Bias may be also resulted from more chance of positive findings published than the negative ones.

The results of our meta-analysis should be interpreted after taking into consideration several inevitable study limitations. First, although we set a comprehensive search strategy for the retrieval of eligible studies, we cannot eliminate that some studies might have been lost. Second, there is language limitation as current meta-analysis only contains English and Chinese literature with some articles possibly published in other languages and not accessible to the international journals. Finally, our results are based on unadjusted ORs while a

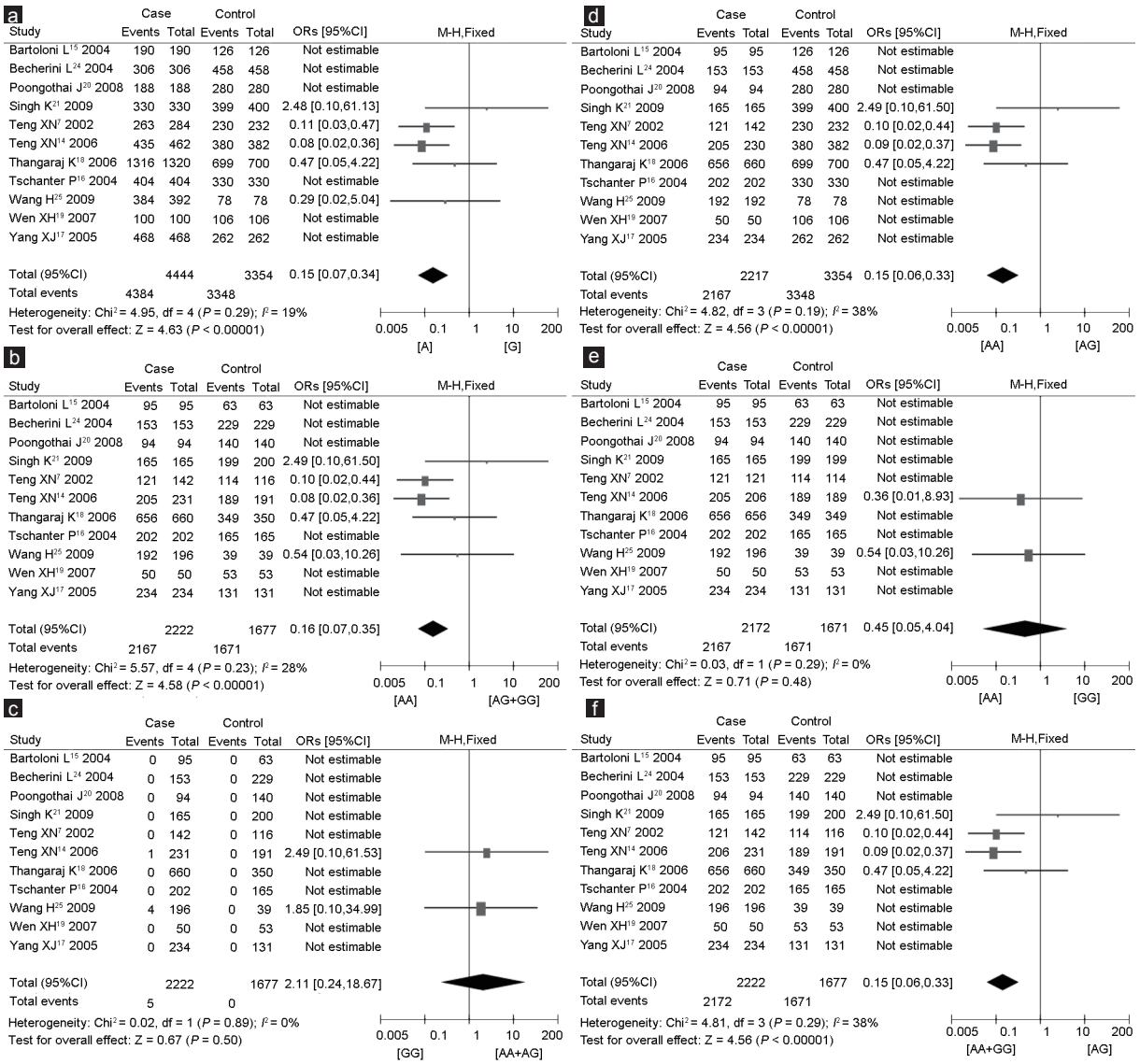

Figure 2: The association of single nucleotide polymorphism 386 with male infertility. A forest plot in (**a**) allele model (A/G), (**b**) dominant model (AA/AG + GG), (**c**) recessive model (AA + AG/GG), (**d**) co-dominant model (AA/AG), (**e**) co-dominant model (AA/GG), (**f**) super-dominant model (AA + GG/AG). The association was indicated as odds ratio (OR) with the corresponding 95% confidence interval (CI). The OR estimate of each study is marked with a solid black square. The size of the square represents the weight that the corresponding study exerts in the meta-analysis. The CIs of pooled estimates are displayed as a horizontal line through the diamond. OR <1 indicates increased risk of male infertility. A: wild type gene and G: mutant gene.

more precise estimation should take into account the effect of multiple confounders such as age and disease severity on the association. Therefore, multicenter, large sample sizes, high-quality case-control or cohort trials are further required to study the exact relationship between *DAZL* polymorphisms and male oligozoospermia or azoospermia.

CONCLUSIONS

Our analysis demonstrated that SNP260 of *DAZL* did not contribute to oligozoospermia or azoospermia in any ethnic subgroups. Instead, SNP386 was correlated to male infertility though this correlation was only found in China with a country-specific and ethnicity-specific manner. However, high-quality trials are further required to study the exact relationship between *DAZL* polymorphisms and male infertility.

AUTHOR CONTRIBUTIONS

All authors have fulfilled all conditions required for authorship. XHZ and PC conceived and designed the experiments. CX, HX, and WHZ performed the electronic search, selected studies, extracted data and performed quality assessment. PC and XW analyzed data and conducted a meta-analysis. XHZ and XHW supervised the research, edited and drafted revisions to the article.

COMPETING INTERESTS

The authors declare no competing interests.

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Supplementary information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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