Polymorphisms in estrogen receptors predict the risk of male infertility: a meta-analysis

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Abstract

Background: Estrogen receptors play an important role in mediating estrogen action on target tissues, and the estrogen is relevant to male infertility. Single nucleotide polymorphisms (SNPs) in estrogen receptors may be associated with the risk of male infertility. A variety of case control studies have been published evaluating this association. However, the accumulated studies have shown inconsistent conclusions.

Methods: To further determine the potential association between the four common SNPs (rs2234693, rs9340799, rs1256049 and rs4986938) in estrogen receptors gene and male infertility, this meta-analysis was performed according to the 10 published case control studies. The odds ratio (OR) and 95% confidence interval (CI) were used to evaluate the strength of the associations.

Results: It was revealed that the sub-group analysis by the ethnicity, for the rs2234693, a significant association in the comparison of CC vs. TT (OR = 0.61, 95% CI: 0.40-0.93), CT vs. TT (OR = 0.67, 95% CI: 0.49-0.93) and CC + CT vs. TT (OR = 0.66, 95% CI: 0.49-0.89) in the Asian population with male infertility. For rs9340799 polymorphism, increased risks were observed for the comparison of AA vs. GG (OR = 1.75, 95% CI: 1.15-2.68) and AA vs. GA + GG (OR = 1.38, 95% CI: 1.02-1.88). For rs1256049 polymorphism, the comparison of the GA vs. GG (OR = 1.52, 95% CI: 1.00-2.31) and AA + GA vs. GG (OR = 1.74, 95% CI: 1.03-2.94), also increased risks present in Asian and Caucasian population, respectively.

Conclusions: The rs2234693C allele was associated with the decreased risk for male infertility; however, the rs9340799AA genotype and the rs1256049GA genotype were associated with an increased risk for male infertility.

Keywords: Male infertility, Polymorphisms, Estrogen receptors
sites [18], and two polymorphisms located in ERα intron 1(T/C transition, rs2234693) and in 50 bp downstream of the former one (G/A transition, rs9340799) have been widely concerned. In addition, the ERβ genes have been described with two silent G/A polymorphisms (rs1256049 and rs4986938) [19]. To date, epidemiological studies have been carried out to evaluate the association between ER polymorphisms and male infertility. However, the results remain inconsistent (Table 1) [5,7,19-26]. In order to get a more precise estimation of the association between polymorphisms in ERs and risk of male infertility, this meta-analysis was performed based on ten eligible previously published studies.

**Methods**

**Identification and eligibility of studies**

To identify all articles that examined the association of ERs polymorphisms with male infertility, a comprehensive systematic bibliographic search through the medical databases PUBMED, attempting to cover all medical papers published between 1950 and 2013, using the following keywords and subject terms: “male infertility”, “polymorphism” and “estrogen receptors” or “ERs”. The synonyms of polymorphism (rs2234693, rs9340799, rs1256049, and rs4986938) were also used as the keywords in the search. The studies were excluded if they were not English language papers or human subject. References in retrieved articles were screened in which case reports, meta-analyses and review articles were excluded. In addition, studies were identified by a manual search of the references lists of reviews and retrieved studies. All the studies were included if they met the following criteria: (I) about the rs2234693, rs9340799, rs1256049, and rs4986938 polymorphisms and male infertility, (II) from a case control study, (III) genotyping methods, (VI) the polymorphism sites, (VII) Hardy–Weinberg equilibrium in the controls, (IX) Hardy–Weinberg equilibrium in the controls. Data were extracted separately for each ethnic groups categorized as Caucasian and Asian. However, no African was identified in this study.

**Data extraction**

Two authors (Tian-Fu Li and Qiu-Yue Wu) extracted all data independently that met the inclusion criteria and reached the consensus for any controversy. The main characteristics of the enrolled studies were listed in the Table 1, including: (I) the first author’s last name, (II) year of publication, (III) ethnicity, (IV) source of control groups (population- or hospital-based controls), (V) genotyping methods, (VI) the polymorphism sites, (VII) characteristics of studies, (VIII) Case/Control counts, (IX) Hardy–Weinberg equilibrium in the controls. Data were extracted separately for each ethnic groups categorized as Caucasian and Asian. However, no African was identified in this study.

**Statistical analysis**

The risk of male infertility associated with the four polymorphisms of the ERα gene was estimated for each study by odds ratio (OR), together with its 95% confidence interval (CI), respectively. The four polymorphisms were evaluated for the associations with male infertility susceptibility based on four genetic models. To contrast, the wild-type homozygote (WW), we first estimated the risk of the rare allele homozygote (RR) and heterozygous (WR) genotypes on infertility, then evaluated the risk of infertility under a dominant model (RR + WR vs. WW). In addition, recessive model associations were also estimated (RR vs. WR + WW). Moreover, stratified analyses were also performed by ethnicity (Asian and Caucasian). The statistical significance of the pooled OR was determined with the Z-test and a P-value of <0.05 was considered significant. Heterogeneity across the studies was evaluated by Chi-square test based on Q test [27] and was considered significant if $P < 0.05$. A fixed-effect model using the Mantel–Haenszel method and a random-effects model using the DerSimonian and Laird method were used to pool the results [28]. In addition, the fixed-effect model was used as well when there was no heterogeneity across results of the studies, or the random-effect model. Moreover, a sensitivity analysis, by which a single study in the meta-analysis was deleted each time to determine the influence of the individual data set to the overall pooled OR, was performed to assess the stability of the results. To test the publication bias, Funnel plots and Egger's linear regression test were applied [29]. Hardy–Weinberg equilibrium in the controls of each study was calculated using a web-based program [30]. All statistical tests for this meta-analysis were performed with STATA version 10.0 (Stata Corporation College Station, TX, USA).

**Results**

**Characteristics of studies**

A total of 10 eligible case control studies with the publication dates ranged from 2002 to 2013 met the prespecified inclusion criteria (shown in the Figure 1), including five studies of Asian population [19-21,23,24] and five studies of Caucasian population [5,7,22,25,26]. To determine the SNPs, two different genotyping methods such as PCR-RFLP [5,19-26] and TaqMan assays [7] were applied. All subjects were received comprehensive andrological examination, and the patients were divided into three types: oligozoospermia (sperm count $< 20 \times 10^6$/mL), azoospermia and oligoasthenoteratozoospermic (OAT). The studies’ exclusion criteria and inclusion criteria were listed in the Table 1. In addition, the sources of controls in these studies were mainly population-based. The distribution of genotypes in the controls of all studies was consistent with Hardy–Weinberg equilibrium except for the
Table 1 Summary of published studies included

| Author       | Year | Race    | Source of control | Method       | Polymorphism sites | Characteristics of study patients                                                                 | Case/control counts | HWE (Control) |
|--------------|------|---------|-------------------|--------------|-------------------|---------------------------------------------------------------------------------------------------|---------------------|---------------|
| Meng [19]    | 2013 | Asian   | PB                | PCR-RFLP     | rs2234693, rs9340799, rs1256049, rs4986938 | Age: 25–38 years (mean age 32.1 ± 5.2 years). Exclusion criteria: abnormal karyotypes, deletions of the Y chromosome, orchitis, varicocele, cryptorchidism, congenital bilateral absence of the vas deferens, hypogonadotropic hypogonadism, and iatrogenic infertility. | TT:83/82, CT:96/126, CC:25/44, AA:151/148, AG:42/89,GG:11/15, GG:103/127, AG:91/102, AA:10/23, GG:155/193, AG:41/48, AA:8/11 | 0.712, 0.793, 0.699, 0.001 |
| Zalata [5]   | 2013 | Caucasian | PB               | PCR-RFLP     | rs2234693, rs9340799 | Inclusion criteria: same ethnic origin (Caucasians). Exclusion criteria: varicocele, hormonal therapy, hypogonadism, smoking, Y chromosome deletions and karyotype abnormalities. The ages were not shown in the article. | TT:33/14, CT:32/27, CC:16/19, AA:28/8, AG:36/32, GG:17/20 | 0.468, 0.389 |
| Ogata [20]   | 2012 | Asian    | PB                | PCR-RFLP     | rs1256049 | Age: 32–52 years (median 41.0 years). Inclusion criteria: no extragenital anomalies, seminal tract obstruction, varicocele, Y chromosomal microdeletion, or retrograde ejaculation; normal karyotypes. | GG:68/64, AG:49/45, AA:8/10 | 0.604 |
| Bianco [7]   | 2011 | Caucasian | PB               | TaqMan assays | rs2234693, rs9340799, rs1256049, rs4986938 | Age: 36.1 ± 6.5 years. Exclusion criteria: chromosome anomalies, azoospermia factor (AZF) microdeletions, smoking, alcoholism, occupation, varicocele, and cryptorchidism. | TT:30/37, CT:93/111, CC:64/68, AA:80/100, AG:79/88, GG:20/8, GG:172/201, AG:15/15, AA:0/0, GG:43/28, AG:60/103, AA:94/85 | 0.468, 0.221, 0.597, 0.712 |
| Safarinejad [21] | 2010 | Asian   | PB                | PCR-RFLP     | rs2234693, rs9340799, rs1256049, rs4986938 | Age: 31.6 ± 4.8 years (range 25–40 years). Inclusion criterion: two years with no reason for their infertility. Exclusion criteria: varicocele or testicular torsion, urinary tract infections, endocrinopathy, karyotype abnormalities, Y-chromosome microdeletions, use of drugs, leukocytospermia, a BMI of 30 kg/m2 or greater. | TG:49/33, CT:70/86, CC:45/45, AA:62/41, AG:77/95, GG:25/28, GG:142/152, AG:21/8, AA:1/4, GG:65/88, AG:62/63, AA:17/21 | 0.486, 0.034, 0.000, 0.132 |
| Lazaros [22] | 2010 | Caucasian | PB               | PCR-RFLP     | rs2234693, rs9340799, rs1256049, rs4986938 | Age: 33.2 ± 6.75 years. Exclusion criteria: hypogonadotropic hypogonadism, obstructive syndromes of the seminal tract, microdeletions of the Y chromosome, karyotypic abnormalities. | TT:6/20, CT:14/40, CC:9/25, AA:5/13, AG:13/43, GG:11/29, GG:26/80, AG:3/5, AA:0/0, GG:7/17, AG:12/36, AA:10/32 | 0.609, 0.652, 0.779, 0.246 |
| Khattri [23] | 2007 | Asian    | PB                | PCR-RFLP     | rs1256049, rs4986938 | Age: 23.24 ± 2.06 years. Exclusion criteria: obstruction, endocrinological defect, injuries, karyotypic abnormality, Y-chromosome microdeletions. | GG:397/231, AG:46/21, AA:0/0 | 0.490 |
| Omrani [24]  | 2005 | Asian    | PB                | PCR-RFLP     | rs1256049, rs4986938 | Exclusion criteria: genetic causes of infertility, such as Klinefelter syndrome or Y-chromosome microdeletions. The ages of patients were no shown in the article. | GG:103/194, AG:17/9, AA:0/1, GG:51/86, AG:57/88, AA:12/30 | 0.023, 0.339 |
| Aschim [25]  | 2005 | Caucasian | PB               | PCR-RFLP     | rs1256049, rs4986938 | Exclusion criteria: Klinefelter syndrome or Y-chromosome microdeletions, a history of cryptorchidism were excluded. The ages of patients were no shown in the article. | GG:92/177, AG:14/8, AA:0/1, GG:11/82, AG:48/79, AA:47/25 | 0.015, 0.394 |
| Kukuvitis [26] | 2002 | Caucasian | PB               | PCR-RFLP     | rs2234693, rs9340799 | Exclusion criteria: any known aetiologies (varicocele, infections of accessory glands, cryptorchidism, homozygous form of β-thalassemia). The ages of patients were no shown in the article. | TT:38/18, CT:38/25, CC:33/21, AA:30/10, AG:45/28, GG:34/26 | 0.083, 0.594 |

PB, Population Based; PCR-RFLP, Polymerase Chain Reaction–restriction Fragment Length Polymorphism; HWE, Hardy–Weinberg equilibrium; BMI, body mass index.
study [19] in rs4986938, the study [21] in rs9340799 and rs1256049, study [24] in rs 1256049 and study [25] in rs1256049, which were tested in the sensitivity analyses.

Quantitative synthesis

Wide variation of four polymorphisms allele frequencies across different ethnicities was observed. For rs2234693, the frequency of T allele was 53.13% (95% CI: 49.74-56.52) in the Asian controls, which was higher than that in Caucasian controls 44.82% (95% CI: 41.48-48.16) as shown in Figure 2A. For rs9340799, the frequency of G allele in the Asian controls (32.45%, 95% CI: 29.27-35.63) was lower than that in Caucasian controls (46.71%, 95% CI: 43.36-50.06) as shown in Figure 2B. In Figure 2C, we could find that the frequency of G allele for the rs1256049 in the Asian controls (87.34%, 95% CI: 85.88-88.81) was lower than which in Caucasian controls (96.92%, 95% CI: 95.82-98.02). In contrast, the frequency of G allele in Caucasian controls (48.46%, 95% CI: 45.32-51.60) was lower than that in Asian group (73.39%, 95% CI: 70.93-75.85) for the rs4986938 in Figure 2D.

For rs2234693 polymorphism, significant differences were observed for the comparison of CC vs. TT, CT vs. TT and CC + CT vs. TT. Sub-group analysis by the ethnicity revealed a significant association in the comparison of CC vs. TT (OR = 0.61, 95% CI: 0.40-0.93, \( P_{\text{heterogeneity}} = 0.670 \), CT vs. TT (OR = 0.67, 95% CI: 0.49-0.93, \( P_{\text{heterogeneity}} = 0.358 \), CC + CT vs. TT (OR = 0.66, 95% CI: 0.49-0.89, \( P_{\text{heterogeneity}} = 0.593 \) and C alleles vs. T alleles (OR = 0.78, 95% CI: 0.64-0.96, \( P_{\text{heterogeneity}} = 0.681 \) in the Asian population, as summarized in Table 2.

For rs9340799 polymorphism, increased risks were observed for the comparison of AA vs. GG and AA vs. GA + GG. Sub-group analysis by ethnicity revealed increased risks (AA vs. GG: OR = 1.75, 95% CI: 1.15-2.68, \( P_{\text{heterogeneity}} = 0.174 \); AA vs. GA + GG: OR = 1.38, 95% CI: 1.02-1.88, \( P_{\text{heterogeneity}} = 0.062 \) in the Caucasian population, also for the AA vs. GA + GG and A alleles vs. G alleles, a significant association was observed in Asian population (OR = 1.93, 95% CI: 1.42-2.62, \( P_{\text{heterogeneity}} = 0.768 \); OR = 1.49, 95% CI: 1.18-1.87, \( P_{\text{heterogeneity}} = 0.375 \) as summarized in Table 3.

For rs1256049 polymorphism, significant differences were observed for the comparison of GA vs. GG, AA + GA vs. GG and AA vs. GA + GG. For the comparison of the GA vs. GG, AA + GA vs. GG, increased risks present in Asian and Caucasian population, respectively (GA vs. GG: OR = 1.52, 95% CI: 1.00-2.31, \( P_{\text{heterogeneity}} = 0.038 \); AA + GA vs. GG: OR = 1.74, 95% CI: 1.03-2.94, \( P_{\text{heterogeneity}} = 0.275 \). All data were concluded in the Table 4. In contrast, a decreased risk was also observed for the comparison AA vs. GA + GG (OR = 0.55, 95% CI: 0.31-0.97, \( P_{\text{heterogeneity}} = 0.818 \)) in Asian population. For the rs4986938, there was
no significant association observed in all comparisons (data were not shown).

**Test of heterogeneity**

Among the four polymorphisms, a significant heterogeneity was apparent among heterozygote comparison for the rs1256049 (GA vs. GG: $P_{\text{heterogeneity}} = 0.047$) (Figure 3). Two studies [7,19] were identified to contribute to substantial heterogeneity, and it was decreased when the study was removed respectively ($P = 0.065$, $P = 0.075$). Sensitivity analysis revealed that the two independent studies [7,23] were the main cause of heterogeneity for the rs1256049. Heterogeneity was decreased when these studies were removed (GA vs. GG: $P_{\text{heterogeneity}} = 0.320$, $I^2 = 14.7\%$). Although the genotype distributions in four studies did not follow Hardy–Weinberg equilibrium, the corresponding pooled ORs were not materially altered by excluding the studies.

**Publication bias**

Begg’s funnel plot and Egger’s test were performed to assess the publication bias of the currently available literature. For the rs2234693, rs9340799 and rs4986938, the shape of the funnel plots did not reveal any evidence of obvious asymmetry in all comparison models. Then, the Egger’s test was used to provide statistical evidence for funnel plot symmetry. The results also did not show any evidence of publication bias. However, for the rs1256049, as shown in the Figure 4, the shape of the funnel plots seemed asymmetrical in the heterozygote and dominant comparisons, suggesting the presence of publication bias. Then, the Egger’s tests were adopted to provide statistical evidence of funnel plot asymmetry. As expected, the results showed obvious evidence of publication bias ($t = 2.53$, $P = 0.044$ for GA vs. GG; $t = 2.71$, $P = 0.035$ for AA + GA vs. GG). To adjust for this bias, a trim-and-fill method developed by Duval and Tweedie [31] was...
Table 2: Stratification analyses of genetic susceptibility of rs2234693 polymorphism to male infertility

| Category | Cases/controls |  |  |  |  |  |  |  |
|----------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|          |                | CC vs. TT OR(95% CI) | P² | I² | CT vs. TT OR(95% CI) | P² | I² | CC + CT vs. TT OR(95% CI) | P² | I² | CC vs. CT + TT OR(95% CI) | P² | I² | C allele vs. T allele OR(95% CI) | P² | I² |
| Total    | 774/841        | 0.72(0.54-0.96)   | 0.282 | 20.1 | 0.74(0.58-0.94) | 0.533 | 0 | 0.73(0.58-0.91) | 0.368 | 7.5 | 0.90(0.71-1.13) | 0.465 | 0 | 0.84(0.71-1.01) | 0.190 | 32.8 |
| RACE     |                |                |       |     |                |       |   |                |       |   |                |       |   |                |       |   |
| Asian    | 368/416        | 0.61(0.40-0.93) | 0.670 | 0 | 0.67(0.49-0.93) | 0.358 | 0 | 0.66(0.49-0.89) | 0.593 | 0 | 0.83(0.58-1.18) | 0.257 | 22.1 | 0.78(0.64-0.96) | 0.681 | 0 |
| Caucasian| 406/425        | 0.83(0.64-1.23) | 0.175 | 39.5 | 0.83(0.57-1.19) | 0.460 | 0 | 0.81(0.58-1.14) | 0.232 | 30 | 0.95(0.70-1.29) | 0.395 | 0 | 0.87(0.64-1.18) | 0.103 | 51.5 |

I²: 0–25, no heterogeneity; 25–50, modest.

P value of Q-test for heterogeneity test.

Bold numbers mean statistically significant results.
Table 3 Stratification analyses of genetic susceptibility of rs9340799 polymorphism to male infertility

| Category | Cases/controls | AA vs. GG | GA vs. GG | AA + GA vs. GG | AA vs. GA + GG | A allele vs. G allele |
|----------|----------------|-----------|-----------|----------------|----------------|-----------------------|
|          |                 | OR(95% CI) | p^2       | OR(95% CI)     | p^2            | OR(95% CI)            | p^2       |
| Total    | 774/841         | 1.67(1.21-2.32) | 0.392 | 3.9 | 1.03(0.76-1.39) | 0.764 | 0 | 1.27(0.96-1.68) | 0.796 | 0 | 1.27(0.96-1.68) | 0.796 | 0 |
| RACE     |                 | 1.63(1.32-2.03) | 0.077 | 49.7 | 1.39(1.13-1.68) | 0.172 | 49.7 |
| Asian    | 368/416         | 1.56(0.93-2.62) | 0.714 | 0 | 0.81(0.49-1.34) | 0.524 | 0 | 1.13(0.71-1.82) | 0.952 | 0 | 0.81(0.49-1.34) | 0.524 | 0 |
| Caucasian| 406/425         | 1.75(1.15-2.68) | 0.174 | 39.7 | 1.17(0.81-1.71) | 0.847 | 0 | 1.35(0.95-1.92) | 0.569 | 0 | 1.35(0.95-1.92) | 0.569 | 0 |

*P value of Q-test for heterogeneity test.
\( \hat{I}^2 \): 0–25, no heterogeneity; 25–50, modest heterogeneity; 50, high heterogeneity.
Bold numbers mean statistically significant results.
Table 4 Stratification analyses of genetic susceptibility of rs1256049 polymorphism to male infertility

| Category      | Cases/Controls | AA vs. GG | GA vs. GG | AA + GA vs. GG | AA vs. GA + GG | A allele vs. G allele |
|---------------|----------------|-----------|-----------|----------------|----------------|----------------------|
|               |                | OR(95% CI) | P*        | I²             | OR(95% CI)     | P*                   | I²                  |
| Total         | 1378/1478      | 0.57(0.32-1.01) | 0.940 | 0  | 1.59(1.12-2.25) | 0.047 | 0.975 | 45.7 | 0.55(0.32-0.96) | 0.920 | 0 | 1.29(0.97-1.72) | 0.068 | 46.9 |
| RACE          |                |            |           |                |                |                      |                    |
| Asian         | 1056/991       | 0.57(0.32-1.01) | 0.853 | 0  | 1.52(1.00-2.31) | 0.038 | 0.64 | 55 | 0.55(0.31-0.97) | 0.818 | 0 | 1.19(0.86-1.65) | 0.067 | 54.4 |
| Caucasian     | 322/487        | 0.64(0.03-15.86) | -       | -  | 1.74(1.03-2.94) | 0.275 | 22.6 | 0.58(0.02-14.38) | - | - | 1.66(0.99-2.77) | 0.372 | 0 |

*P value of Q-test for heterogeneity test.
*bRandom-effects model was used when a P value, 0.05 for heterogeneity test; otherwise, fixed-effects model was used.

I²: 0-25, no heterogeneity; 25-50, modest heterogeneity; 50, high heterogeneity.

Bold numbers mean statistically significant results.
implemented. Trimming was based on fixed-effects model, and the adjusted estimates obtained by using the random effects model were ORs of 1.17 (0.78-1.74) for GA vs. GG and 1.08 (0.75-1.54) for AA + GA vs. GG in the Figure 5. Although Meta-analysis with or without the trim-and-fill method also ends up with same conclusions, but the ORs were not statistically significant difference. So it was indicated that the results of these studies were not statistically robust.

Discussion

The present meta-analysis, including 1568 cases and 1602 controls from 10 case control studies, explored the association between the ERs polymorphisms and male infertility. The results indicated that rs2234693C allele was associated with decreased risk of the male infertility, particularly in the Asian population. In contrast, rs9340799AA genotype was observed as a risk factor for infertility in both Asian and Caucasian population, and rs1256049GA genotype was associated with an increased risk for developing male sterility. However, the rs4986938 polymorphism was not associated with male infertility. In addition, we tried to find the data in the available database, such as PUBMED [32], National Human Genome Research Institute GWAS Catalog [33] and GWAS Central [34], but we found no relevant genome-wide association (GWAS) study about these four polymorphisms.

Estradiol has been reported as a survival factor for germ cells [11], involving in the induction of oxidative DNA damage, and the aberrant level of estrogen may lead to impaired sperm production [35-37]. It has been shown that free radicals inhibit steriodogenesis by interfering with cholesterol transport to the mitochondria and/or the catalytic function of P450 enzymes, which leads to an increase in lipid per oxidation and decline in the antioxidant barrier [38]. Moreover, estrogens can regulate mitochondrial function by increasing nuclear respiratory factor-1 (NRF-1) expression [5]. Specifically, estradiol stimulates mitochondrial function through a genomic mechanism of ER action involving direct ERS and ERβ interaction with an oestrogen response element in the NRF-1 promoter [39]. In vivo knockdown experiments have indicated that estradiol stimulates NRF-1 transcription and consequently increases mitochondrial biogenesis through ERS activity but not through ERβ activity in MCF-7 breast cells [40]. This findings indicates that ERS polymorphisms

![Figure 3 Forest plot for the overall association between rs1256049 polymorphism and male infertility for random effects.](http://www.rbej.com/content/12/1/79)
can increase mitochondrial activity via NRF-1 transcription in human ejaculated spermatozoa, presenting them with high motility [22].

The mechanisms behind altered ERs function in subjects with polymorphisms remain unclear. The polymorphism rs1256049 located at the splice acceptor site just prior to exon 8 in ERβ [41] and may potentially affect the splicing of this exon, leading to proteins with different properties than the wild-type ERβ [42,43]. In addition, studies have reported the polymorphism could also have a direct effect through changing the nucleotide sequence and thereby the secondary structure of the ERβ mRNA, possibly leading to changes the function of mRNA [44,45]. It has been reported that ERα gene polymorphisms (rs2234693 and rs9340799) may modulate the effect of oestradiol on CYP19, which encodes aromatase expression, disrupting the gene causes a decline in sperm numbers and loss of male infertility [46,47].

The precise role of estrogen receptors in male fertility status is understood. Some findings suggest that specific polymorphisms of the ERα, and ERβ genes which confer a lower sex hormone binding globulin (SHBG) and thus a stronger unbound estrogen effect, may adversely affect human spermatogenesis [48,49]. SHBG is involved in both delivering reproductive hormones to target tissues and controlling the concentration of androgens and estrogens in the serum and tissues [50]. Pavlovich et al. [51] demonstrated that infertile men with severe oligozoospermia had significantly lower T (testosterone) and higher E2 (estradiol) concentrations than fertile control subjects, resulting in an elevated T/E2 ratio.

Identifying the source of heterogeneity is one of the most important goals of the meta-analysis. Thus, we stratified the studies only according to ethnicity (because the sources of the controls were selected through population-based, and the method used was the only one different).
Stratified analysis by ethnicity revealed that there was no difference between the European population and Asian population, suggesting that different ethnicities and environmental exposures may have no influence on the susceptibility of male infertility, and more studies should be accumulated to reveal the difference. In addition, for the rs1256049, sensitivity analysis revealed that the three independent studies [7,22,23] were the main source of heterogeneity. Heterogeneity was decreased when these studies were removed. For these three studies, the sample size was not sufficient and the numbers of rs1256049AA genotype was both zero. These two points may be the main reason for the heterogeneity in the performed analysis. For the rs1256049, there was obvious evidence of publication bias. As the same with heterogeneity, the numbers of the cases and controls of the wild-type homozygote in these three studies [7,22,23] were too small to keep the results statistically robust, so it maybe the key factor for the bias. Using a proper and representative subject is very important in reducing bias in such genotype association studies.

There are still some limitations in this meta-analysis. Firstly, there were only ten literatures enrolled in this meta-analysis, the sample size was not big enough to have substantial power exploring the real association. Secondly, the detailed information (such as life-style, age, and work) could not be traced, so that our unadjusted estimates should be confirmed by further studies. In addition, an individual with a clinical disorder was not a result of the single gene that is disrupted, but that the genetic disruption was embedded within the context of that individual’s entire genome and environment exposure [52]. In fact, some other genes related to fertility could also play an important role in spermatogenesis.

**Figure 5** Begg’s funnel plot of publication bias test for the rs1256049 using the trim-and-fill method. (A) GA vs. GG. (B) AA + GA vs. GG. Each point represents a separate study for the indicated association. Log (OR), natural logarithm of OR. Horizontal line means effect size. The adjusted estimates obtained by using the random effects model for GA vs. GG and AA + GA vs. GG.
Conclusions
In summary, this meta-analysis suggested that the rs2334693C allele was the protective factor for male infertility, the rs9340799AA genotype was associated with an increased risk for infertility, and the rs1256049GA genotype was also the negative factor.

Additional file

Additional file 1: Text S1. The reasons for exclusion of the articles which were shown in Figure 1.

Abbreviations
CI: Confidence interval; E2: Estradiol; ERs: Estrogen receptors; NR: Nuclear receptor; NRF-1: Nuclear respiratory factor-1; OR: Odds ratio; PCR-RFLP: Polymerase chain reaction–restriction fragment length polymorphism; SHBG: Sex hormone binding globulin; SNP: Single nucleotide polymorphism; T: Testosterone; vs.: Versus.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
TFL and XYX conceived and designed the experiments. TFL and QYW analyzed the data. TFL and XJC performed the experiments. TFL and QYW, NL, CZ, and WWL, QYW, NL, CZ, and XYX contributed to the acquisition of data. TFL and XYX performed the statistical analysis. TFL and QYW contributed to the drafting and critical review of the manuscript.

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