Association of polymorphisms in estrogen receptors (*ESR1* and *ESR2*) with male infertility: a meta-analysis and systematic review

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Abstract

Purpose Estrogens play an important role in male reproduction via interacting with estrogen receptors (ERs), whose expression can be regulated by the polymorphisms in different regions of *ESR1* and *ESR2* genes. However, results from published studies on the association between four well-characterized polymorphisms (*PvuII*, *XbaI*, *RsaI*, and *AluI*) in the gene of ERs (*ESR1* and *ESR2*) and male infertility risk are inconclusive.

Methods To investigate the strength of relationship of *PvuII* and *XbaI* in *ESR1* and *RsaI* and *AluI* in *ESR2* with male infertility, we conducted a meta-analysis of 12 eligible studies with odds ratio (OR) and its corresponding 95 % confidence intervals (95 % CI).

Results Overall, *ESR1 PvuII* and *ESR2 RsaI* polymorphisms were significantly associated with male infertility risk. The subgroup analyses by ethnicities demonstrated that in Asians, *ESR1 PvuII*, *XbaI* and *ESR2 RsaI* polymorphisms were significantly associated with a decreased infertility risk, while in Caucasians both *ESR1 PvuII* and *ESR2 RsaI* polymorphisms increased the susceptibility to male infertility. As for *ESR2 AluI* polymorphism, no significant association was detected in either overall analysis or subgroup analyses by ethnicities/genotyping methods.

Conclusions This meta-analysis suggested that polymorphisms in the genes of ERs (*ESR1* and *ESR2*) may have

differential roles in the predisposition to male infertility according to the different ethnic backgrounds. Further well-designed and unbiased studies with larger sample size and diverse ethnic backgrounds should be conducted to verify our findings

Keywords Estrogen receptor · Polymorphism · Male infertility · Meta-analysis

Introduction

Infertility is a major health problem affecting one-sixth of couples worldwide, whose cause is shared equally between male and female partners [1, 2]. Despite the consistent advance in the diagnostic workup of male infertility, the etiology and pathogenesis in about 30 % of cases are yet unknown, and their conditions are considered as idiopathic infertility [3]. Genetic abnormalities have been identified as one of the major contributing factors of male infertility [4].

Although estrogens have been conventionally regarded as female steroid hormones, their profound effects on male productive systems have been widely investigated and well documented [5]. In males, the estrogens are synthesized from testosterone through the action of aromatase cytochrome P450 in testes [6], and the concentration of estrogens in semen is higher than that in serum of women [7]. However, the role of estrogens on male infertility is still a matter of controversy. On one hand, estrogen deficiency can lead to reduced sperm production and sperm motility in humans [8]; on the other hand, estrogen excess during the adulthood can deteriorate sperm production and maturation [9].

The physiological responses to estrogens are mediated by estrogen receptors (ERs), which consist of three subtypes: ER α , ER β and ER γ [10]. Of these three isoforms, ER α and

Capsule The differential association of polymorphisms in estrogen receptors (*PvuII*, *XbaI*, *RsaI*, and *AluI*) with male infertility was summarized.

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ER β have been well investigated, while ER γ is an emerging ER similar to ER β , which was detected in various cellular models, including human spermatozoa [11]. ER α is a 595 amino acid protein encoded by *ESR1* which is located on chromosome 6q25 [12, 13], while ER β is a 530 amino acid protein encoded by *ESR2* on chromosome 14q23-24 with a total size of 40 kb [14, 15]. Both receptors are expressed in human testicular germ cells at different stages of spermatogenesis [16], and exert important functions on male reproductive capability via the interaction with estrogens [5].

Genetic screening for *ESR1* and *ESR2* gene locus has demonstrated several single nucleotide polymorphisms (SNPs). For *ESR1*, the two most studied SNPs are *PvuII* (397T>C, rs2234693) and *XbaI* (351G>A, rs9340799), which were located in intron 1 and separated by 46 bp. The *XbaI* polymorphism is caused by a A-to-G transition at position +397, whereas the *PvuII* polymorphism is resulted from a T-to-C transition upstream of the *XbaI* polymorphic site [17]. As for *ESR2*, two silent G-to-A SNPs, *RsaI* (1082G>A, rs1256049) and *AluI* (1730G>A, rs4986938), have been widely investigated [18].

Since the first study reported by Kukuvtis and colleagues on the association between polymorphisms in the genes of ERs (*ESR1* and *ESR2*) and risk of male infertility [19], many similar investigations in different ethnicities have been conducted [20–34]; however, the results of the published studies are inconclusive and even controversial. Thus, we conduct this meta-analysis to explore the exact association of the four common SNPs (*PvuII*, *XbaI*, *RsaI*, and *AluI*) in *ESR1* and *ESR2* with male infertility.

Materials and methods

Identification of eligible studies

This meta-analysis was conducted and reported in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines [35]. A comprehensive search of PUBMED and EMBASE was performed until December 1, 2013 to identify all eligible studies examining the association of the four common SNPs (*PvuII*, *XbaI*, *RsaI*, and *AluI*) in ERs with male infertility risk. No restrictions were placed on language, and only published studies with full-text articles were included. To search and include as many related studies as possible, we used different combinations of the following medical subject headings and key words: estrogen receptors, *ESR1*, or *ESR2*; polymorphism or variant; male infertility or spermatogenic failure. Furthermore, the reference lists of reviews and retrieved articles were manually screened for additional studies.

Inclusion and exclusion criteria

Studies identified from the above mentioned databases (PUBMED and EMBASE) were screened by two independent authors (Yu-Zheng Ge and Lu-Wei Xu) according to the following predesigned inclusion criteria: 1) case-control design; 2) evaluating the correlation of the four SNPs (*PvuII*, *XbaI*, *RsaI*, and *AluI*) with male infertility risk; 3) providing sufficient data to calculate the odds ratio (OR) and its corresponding 95 % confidence interval (CI). When several studies with overlapping data were eligible, those with smaller sample size or less reliability were excluded. Furthermore, studies without detailed information were excluded, after the efforts to extract data from the original paper or contact the corresponding authors failed.

Data extraction

All data from eligible studies were extracted by two reviewers (Yu-Zheng Ge and Zheng Xu) independently and in duplicate according to the predesigned data-collection form. The following information was extracted: last name of first author, publication year, country of origin, ethnicity, genotyping method, case definition and Hardy-Weinberg equilibrium (HWE). When HWE was not reported in controls, an online program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>) was applied to test the HWE by χ^2 test for goodness of fit [36]. Discrepancies occurring during the process of studies selection and data extraction were resolved by discussion with a third reviewer (Wen-Cheng Li), and consensus on each item was achieved at last.

Statistical analysis

The strength of association between the four polymorphisms in ERs and male infertility risk was measured by OR with its corresponding 95 % CI. The pooled ORs were calculated for the following five genetic models (1. Allelic model: A allele vs. a allele; 2. homozygote comparison: AA vs. aa; 3. heterozygote comparison: Aa vs. aa; 4. dominant model: AA + Aa vs. aa; and 5. recessive model: AA vs. Aa + aa. A: variant allele, a: wild allele, and allele A, T, G and G were considered as wild alleles for *PvuII*, *XbaI*, *RsaI*, and *AluI*, respectively). Stratified analyses were also conducted based on ethnicities and genotyping methods. The statistical significance of the pooled OR was assessed with the Z test and $P < 0.05$ was considered significant.

Chi-square based Q test was conducted to measure the heterogeneity between eligible studies, and the existence of heterogeneity was considered significant if $P < 0.10$ [37]. When the between-study heterogeneity was absent, a fixed-effect model (Mantel-Haenszel method) was used to pool the data from different studies [38]; otherwise, a random-effects

model (DerSimonian and Laird method) was applied [39]. Sensitivity analyses were performed to identify each individual study's effect on pooled results and test the reliability of results by deleting a single study each time [40]. To determine the presence of publication bias, Begg's funnel plot and Egger's linear regression test were conducted, and $P < 0.05$ was considered significant [41, 42].

All statistical tests for this meta-analysis were performed with STATA software (version 10.0; Stata Corporation, College Station, Texas, USA) and Review Manager (version 5.0; Cochrane Collaboration, Oxford, UK).

Results

Characteristics of eligible studies

Records identified from the databases were primarily screened by titles and abstracts, and 16 full-text articles were retrieved for further assessment of the eligibility. Among those studies, four were excluded for: 1) without control [20]; 2) not targeted SNPs [24]; 3) data overlapped with others [25] and 4) subjects recruited from childhood cancer survivors [30]. After all, a total of 12 eligible articles were included in this meta-analysis, and the detailed screening process was shown in Fig. 1, which was modified according to the PRISMA Statement [35]. Among these 12 eligible studies, 7, 6, 9, and 9 studies were pooled for the analysis of *PvuII*, *XbaI*, *RsaI*, and *AluI* polymorphisms, respectively. As for ethnicities, four were studies of Caucasians [19, 21, 22, 28, 34], six studies were of Asians [23, 26, 27, 29, 32, 33], and one studies of mixed ethnicity [31]. To determine the SNPs, two different genotyping methods such as RFLP-PCR [19, 21–23, 26, 29, 33, 34] and TaqMan PCR [27, 28, 31, 32] were applied (Table 1).

Quantitative data synthesis

Association of ESR1 PvuII polymorphism with male infertility

A total of seven studies with 840 cases and 936 controls were included to examine the association between *ESR1 PvuII* polymorphism and male infertility risk, including three Caucasian studies [19, 22, 28, 34], 2 Asian studies [29, 33], and 1 study with mixed population [31]. Overall, the *PvuII* polymorphism in *ESR1* was associated with a significantly decreased risk of male infertility in heterozygote comparison (CT vs. TT, OR=0.78, 95 % CI: 0.62–0.98, $P_{\text{heterogeneity}} = 0.415$; Fig. 2). Further subgroup analyses based on ethnicities and genotyping methods demonstrated that *ESR1 PvuII* polymorphism was significantly associated with an decreased risk in Asian males (C allele vs. T allele, OR=0.78, 95 % CI: 0.64–

0.96; CC vs. TT, OR=0.61, 95 % CI: 0.40–0.93; CT vs. TT, OR=0.67, 95 % CI: 0.49–0.93; CC + CT vs. TT, OR=0.66, 95 % CI: 0.49–0.90) and an increased risk in Caucasians (CC vs. CT + TT: OR=1.52, 95 % CI: 1.05–2.22). With regard to the subgroup analysis by genotyping methods, a significant association was detected in RFLP-PCR genotyping method under both heterozygote comparison (CT vs. TT: OR=0.71, 95 % CI: 0.55–0.92) and dominant model (CC + CT vs. TT, OR=0.77, 95 % CI: 0.61–0.98), the detailed results was presented in Table 2.

Association of ESR1 XbaI polymorphism with male infertility

Six studies including 736 infertile men and 841 healthy fertile controls were identified for evaluating the relationship between *XbaI* polymorphism in *ESR1* and male infertility. Overall, as shown in Table 3, no significant association was detected between *ESR1 XbaI* polymorphism and infertility susceptibility in males. Further stratification analysis by ethnicities demonstrated a significant association in Asian population in allelic model (G allele vs. A allele, OR=0.67, 95 % CI: 0.54–0.85, $P_{\text{heterogeneity}} = 0.375$), heterozygote comparison (AG vs. AA, OR=0.49, 95 % CI: 0.36–0.68, $P_{\text{heterogeneity}} = 0.660$; Fig. 3) and dominant model (GG + GA vs. AA, OR=0.52, 95 % CI: 0.38–0.71, $P_{\text{heterogeneity}} = 0.768$), while in Caucasian population, no significant association was detected. In the subgroup analysis based on genotyping method, no significant association was found in all genetic models.

Association of ESR2 RsaI polymorphism with male infertility

Data from nine case-control studies involving 1,418 infertile cases and 1,601 healthy fertile controls were pooled together to explore the potential association between *ESR2 RsaI* polymorphism with male infertility. The overall results indicated that variant A allele of *ESR2 RsaI* polymorphism was associated with a lower risk for male infertility in homozygote comparison (AA vs. GG, OR=0.56, 95 % CI: 0.32–0.98, $P_{\text{heterogeneity}} = 0.964$) and recessive model (AA vs. AG + GG, OR=0.54, 95 % CI: 0.31–0.93, $P_{\text{heterogeneity}} = 0.954$), while an increased risk in heterozygote comparison (AG vs. GG, OR=1.54, 95 % CI: 1.06–2.24, $P_{\text{heterogeneity}} = 0.025$; Fig. 4). The further subgroup analysis based on ethnicities demonstrated that *ESR2 RsaI* polymorphism was significantly associated with a decreased infertility risk for Asian population in homozygote comparison (AA vs. GG, OR=0.56, 95 % CI: 0.31–0.98, $P_{\text{heterogeneity}} = 0.914$) and recessive model (AA vs. AG + GG, OR=0.54, 95 % CI: 0.31–0.94, $P_{\text{heterogeneity}} = 0.895$) and an increased risk for Caucasian men in Allelic model (A allele vs. G allele, OR=2.35, 95 % CI: 1.14–4.84, $P_{\text{heterogeneity}} = 0.682$), heterozygote comparison (AG vs. GG, OR=2.87,

Fig. 1 Flow diagram for study selection. Description: a total of 12 studies were included in this meta-analysis and systematic review after a comprehensive study selection

95 % CI: 1.32–6.22, $P_{\text{heterogeneity}}=0.501$) and dominant model (AA + AG vs. GG, OR=2.65, 95 % CI: 1.24–5.63, $P_{\text{heterogeneity}}=0.585$). As for the subgroup analysis by

genotyping methods, the results indicated that the examined SNP was associated with a decreased risk with RFLP-PCR genotyping method in homozygote comparison (AA vs. GG,

Table 1 Characteristics of eligible studies included in the meta-analysis

Author	Year	Country	Ethnicity	Genotyping method	Investigated SNPs	Case definition
Kukuvitis	2002	Greece	Caucasian	RFLP-PCR	<i>PvuII</i> , <i>XbaI</i>	Oligospermia or azoospermia
Aschim	2005	Sweden	Caucasian	RFLP-PCR	<i>RsaI</i> , <i>AluI</i>	Oligozoospermia or azoospermia
Galan	2005	Spain	Caucasian	RFLP-PCR	<i>PvuII</i> , <i>AluI</i>	Oligozoospermia or azoospermia
Omrani	2006	Iran	Asian	RFLP-PCR	<i>RsaI</i> , <i>AluI</i>	Azoospermia or severe oligozoospermia
Khattari	2009	India	Asian	RFLP-PCR	<i>RsaI</i> , <i>AluI</i>	Azoospermia, oligoasthenozoospermia or oligoasthenoteratozoospermia
Su	2010	China	Asian	TaqMan PCR	<i>RsaI</i> , <i>AluI</i>	Oligozoospermia or azoospermia
Lazaros	2010	Greece	Caucasian	TaqMan PCR	<i>PvuII</i> , <i>XbaI</i> , <i>RsaI</i> , <i>AluI</i>	Oligospermia
Safarinejad	2010	Iran	Asian	RFLP-PCR	<i>PvuII</i> , <i>XbaI</i> , <i>RsaI</i> , <i>AluI</i>	Oligoasthenoteratozoospermia
Bianco	2011	Brazil	Mixed	TaqMan PCR	<i>PvuII</i> , <i>XbaI</i> , <i>RsaI</i> , <i>AluI</i>	Azoospermia or severe oligozoospermia
Ogata	2012	Japan	Asian	TaqMan PCR	<i>RsaI</i>	Azoospermia or severe oligozoospermia
Zalata	2013	Egypt	Caucasian	RFLP-PCR	<i>PvuII</i> , <i>XbaI</i>	Oligoasthenoteratozoospermia
Meng	2013	China	Asian	RFLP-PCR	<i>PvuII</i> , <i>XbaI</i> , <i>RsaI</i> , <i>AluI</i>	Oligozoospermia or azoospermia

SNP single nucleotide polymorphism, PCR polymerase chain reaction, RFLP restriction fragment length polymorphism

Fig. 2 Forest plot for the relationship of *ESR1* PvuII polymorphism with male infertility (CT vs. TT) stratified by ethnicity. For each study, the estimate of OR and its 95 % CI is plotted with a box and a horizontal line. Filled diamond pooled OR and its 95%CI

OR=0.49, 95 % CI: 0.25–0.97, $P_{\text{heterogeneity}}=0.973$) and recessive model (AA vs. AG + GG, OR=0.47, 95 % CI: 0.24–0.91, $P_{\text{heterogeneity}}=0.972$) while a higher risk in heterozygote comparison (AG vs. GG, OR=1.85, 95 % CI: 1.05–3.27, $P_{\text{heterogeneity}}=0.006$) (Table 4).

Association of *ESR2* AluI polymorphism with male infertility

A total of nine eligible studies with 1,397 infertile cases and 1,577 healthy fertile controls were included for the analysis. Of these nine studies, six were conducted in Asian population

Table 2 meta-analysis results of the association of *ESR1* PvuII polymorphism with male infertility

Category	Num ^a	C allele vs. T allele		CC vs. TT		CT vs. TT		CC + CT vs. TT		CC vs. CT + TT	
		OR(95%CI)	P ^b	OR(95%CI)	P ^b	OR(95%CI)	P ^b	OR(95%CI)	P ^b	OR(95%CI)	P ^b
Total	7	1.01(0.82,1.24)	0.051	1.01(0.69,1.48)	0.089	0.78(0.62,0.98)^c	0.415	0.83(0.67,1.03)	0.177	1.11(0.90,1.38)	0.217
Ethnicity											
Asian	2	0.78(0.64,0.96)^c	0.681	0.61(0.40,0.93)^c	0.670	0.67(0.49,0.93)^c	0.358	0.66(0.49,0.90)^c	0.593	0.83(0.58,1.18)	0.257
Caucasian	4	1.21(0.90,1.62)	0.208	1.42(0.90,2.23)	0.381	0.83(0.56,1.24)	0.343	1.02(0.71,1.46)	0.227	1.52(1.05,2.22)^c	0.628
Mixed	1	1.08(0.82,1.43)	–	1.61(0.64,2.10)	–	1.03(0.59,1.80)	–	1.08(0.64,1.83)	–	1.13(0.75,1.72)	–
Genotyping method											
RFLP-PCR	5	1.00(0.75,1.32)	0.022	0.99(0.59,1.66)	0.037	0.71(0.55,0.92)^c	0.392	0.77(0.61,0.98)^c	0.127	1.11(0.85,1.45)	0.082
TaqMan	2	1.08(0.84,1.40)	0.974	1.17(0.69,1.98)	0.961	1.06(0.65,1.74)	0.847	1.10(0.67,1.76)	0.883	1.12(0.77,1.64)	0.926

^a Num, number of eligible studies

^b P value of Q test for heterogeneity test

^c Statistically significant results (in bold)

Table 3 meta-analysis results of the association of *ESR1 XbaI* polymorphism with male infertility

Category	Num ^a	G allele vs. A allele		GG vs. AA		GA vs. AA		GG + GA vs. AA		GG vs. GA + AA	
		OR(95%CI)	P ^b	OR(95%CI)	P ^b	OR(95%CI)	P ^b	OR(95%CI)	P ^b	OR(95%CI)	P ^b
Total	6	0.88(0.65,1.20)	0.002	0.87(0.50,1.50)	0.026	0.82(0.54,1.25)	0.014	0.74(0.50,1.10)	0.017	0.92(0.53,1.60)	0.003
Ethnicity											
Asian	2	0.67(0.54,0.85)^c	0.375	0.64(0.38,1.07)	0.714	0.49(0.36,0.68)^c	0.660	0.52(0.38,0.71)^c	0.768	0.59(0.30,1.16)	0.177
Caucasian	3	1.10(0.55,2.19)	0.004	1.20(0.30,1.48)	0.005	0.85(0.48,1.50)	0.317	0.94(0.39,2.24)	0.053	1.10(0.67,1.83)	0.060
Mixed	1	0.93(0.70,1.25)	—	0.81(0.43,1.54)	—	1.02(0.67,1.55)	—	0.97(0.66,1.44)	—	0.50(0.27,0.92)^c	—
Genotyping method											
RFLP-PCR	4	0.85(0.54,1.35)	0.001	0.90(0.38,2.10)	0.005	0.79(0.44,1.40)	0.010	0.67(0.40,1.14)	0.019	0.98(0.47,2.07)	0.004
TaqMan	2	0.95(0.73,1.24)	0.755	0.85(0.48,1.50)	0.785	0.99(0.67,1.47)	0.688	0.96(0.66,1.39)	0.854	0.83(0.28,2.48)	0.040

^a Num, number of eligible studies^b P value of Q test for heterogeneity test^c Statistically significant results (in bold)

[23, 26, 27, 29, 33], three in Caucasians [21, 22, 28], and one in the mixed population [31]. Of note, the study reported by Su et al. only presented allelic distribution of two groups [27]; therefore, it was merely included in the allelic model. Overall, as presented in Table 5, no significant association was detected about the association of studied SNP with male infertility risk. Stratified analyses based on ethnicities and genotyping

methods also failed to detect the significant association between *ESR2 AluI* polymorphism and male infertility risk.

Heterogeneity test and sensitivity analysis

During the meta-analysis, the significant between-study heterogeneity was observed (Tables 2, 3, 4 and 5). To

Fig. 3 Forest plot for the association between *ESR1 XbaI* polymorphism with male infertility (AG vs. AA) stratified by ethnicity. For each study, the estimate of OR and its 95 % CI is plotted with a box and a horizontal line. Filled diamond pooled OR and its 95%CI

Fig. 4 Forest plot for the correlation of *ESR2 RsaI* polymorphism with male infertility (AG vs. GG) stratified by ethnicity. For each study, the estimate of OR and its 95 % CI is plotted with a box and a horizontal line. Filled diamond pooled OR and its 95%CI

explore the source of heterogeneity, stratification analyses by ethnicities and genotyping methods were conducted. Furthermore, sensitivity analyses were performed to explore the influence of each individual study on the overall results by deleting one single study each time from the pooled analysis. The results indicated the three studies contributed to the main source of between-study heterogeneity [33, 23, 34]. In addition, no single study was found to have the substantial power to affect the pooled ORs significantly (Data not shown).

Publication bias

To assess the publication bias of the currently available literature, both Begg's funnel plot and Egger's test were performed. The shapes of the funnel plots did not reveal any evidence of obvious asymmetry in all comparison models (data not shown). 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 (Table 6).

Discussion

Male infertility is a major global health problem, which is contributory in approximately 50 % of couples unable to achieve pregnancy after regular intercourses over 1 year [1, 26]. Many efforts have been made to explore the potential biomarkers with clear-cut diagnostic and prognostic values, including the SNPs in the genes of ERs (*ESR1* and *ESR2*). However, due to the relative small sample size, no clear consensus has reached on the relationship of four common SNPs (*PvuII*, *XbaI*, *RsaI*, and *AluI*) in ERs with male infertility risk; therefore this meta-analysis was conducted. In the present study, we provide evidence that two well-characterized SNPs (*PvuII* and *XbaI*) of *ESR1* were associated with a significantly decreased risk for male infertility, especially in Asian population with RFLP-PCR genotyping method; in case of *ESR2 RsaI* polymorphism, diverse results were yielded that it was significantly with a lower risk at homozygote and recessive level while an increased risk at heterozygote level; as for *ESR2 RsaI* polymorphism, no significant association with male infertility risk was detected.

Table 4 meta-analysis results of the association of *ESR2* *RsaI* polymorphism with male infertility

Category	A allele vs. G allele			AA vs. GG			AG vs. GG			AA + AG vs. GG			AA vs. AG + GG		
	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b
Total	9	1.44(0.99,2.07)	<0.001	6	0.56(0.32,0.98)^c	0.964	8	1.54(1.06,2.24)^c	0.025	8	1.39(0.99,1.95)	0.044	6	0.54(0.31,0.93)^c	0.954
Ethnicity															
Asian	6	1.37(0.87,2.14)	<0.001	5	0.56(0.31,0.98)^c	0.914	5	1.44(0.91,2.27)	0.022	5	1.27(0.85,1.90)	0.043	5	0.54(0.31,0.94)^c	0.895
Caucasian	2	2.35(1.14,4.84)^c	0.682	1	0.64(0.03,15.9)	–	2	2.87(1.32,6.22)^c	0.501	2	2.65(1.24,5.63)^c	0.585	1	0.58(0.02,14.4)	–
Mixed	1	1.16(0.56,2.41)	–	0	–	–	1	1.17(0.56,2.46)	–	1	1.17(0.56,2.46)	–	0	–	–
Genotyping method															
RFLP-PCR	6	1.61(0.97,2.66)	<0.001	5	0.49(0.25,0.97)^c	0.973	5	1.85(1.05,3.27)^c	0.006	5	1.61(0.96,2.70)	0.011	5	0.47(0.24,0.91)^c	0.972
TaqMan	3	1.02(0.72,1.43)	0.644	1	0.75(0.28,2.03)	–	3	1.12(0.74,1.69)	0.760	3	1.08(0.72,1.61)	0.707	1	0.75(0.31,1.96)	–

^a N, number of eligible studies^b P value of Q test for heterogeneity test^c Statistically significant results (in bold)**Table 5** meta-analysis results of the association of *ESR2* *AluI* polymorphism with male infertility

Category	A allele vs. G allele			AA vs. GG			AG vs. GG			AA + AG vs. GG			AA vs. AG + GG		
	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b	N ^a	OR(95%CI)	P ^b
Total	9	0.99(0.89,1.12)	0.620	8	1.03(0.79,1.34)	0.743	8	1.01(0.80,1.27)	0.095	8	1.19(0.84,1.69)	<0.001	8	1.06(0.83,1.36)	0.195
Ethnicity															
Asian	5	0.99(0.86,1.15)	0.250	4	0.94(0.65,1.35)	0.760	4	1.22(0.99,1.52)	0.601	4	1.66(0.93,2.94)	<0.001	4	0.84(0.59,1.20)	0.752
Caucasian	3	0.93(0.73,1.19)	0.958	3	0.88(0.52,1.48)	0.783	3	0.89(0.61,1.28)	0.593	3	0.88(0.63,1.25)	0.801	3	0.95(0.60,1.52)	0.536
Mixed	1	1.10(0.83,1.46)	–	1	1.55(0.89,2.73)	–	1	0.59(0.38,0.91)^c	–	1	0.80(0.54,1.18)	–	1	2.01(1.19,3.38)^c	–
Genotyping method															
RFLP-PCR	7	0.98(0.86,1.12)	0.481	6	0.93(0.68,1.27)	0.903	6	1.14(0.94,1.38)	0.447	6	1.34(0.88,2.04)	<0.001	6	0.88(0.66,1.19)	0.759
TaqMan	2	1.05(0.81,1.36)	0.472	2	1.35(0.82,2.23)	0.266	2	0.62(0.41,0.93)^c	0.598	2	0.79(0.55,1.15)	0.982	2	1.61(0.83,1.36)	0.112

^a N, number of eligible studies^b P value of Q test for heterogeneity test^c Statistically significant results (in bold)

Table 6 Statistical analyses of publication bias for estrogen receptors polymorphisms

Category	Allelic Model	Homozygote Comparison	Heterozygote Comparison	Dominant Model	Recessive Model
<i>ESR1 PvuII</i>	C vs. T allele	CC vs. TT	CT vs. TT	CC + CT vs. TT	CC vs. CT + TT
Begg's test	0.230	0.133	0.230	0.133	0.368
Egger's test	0.134	0.126	0.344	0.117	0.311
<i>ESR1 XbaI</i>	G vs. A allele	GG vs. AA	GA vs. AA	GG + GA vs. AA	GG vs. GA + AA
Begg's test	1.000	0.707	1.000	0.707	0.452
Egger's test	0.423	0.382	0.766	0.641	0.323
<i>ESR2 RsaI</i>	A vs. G allele	AA vs. GG	AG vs. GG	AA + AG vs. GG	AA vs. AG + GG
Begg's test	0.118	0.707	0.108	0.063	0.707
Egger's test	0.291	0.413	0.066	0.056	0.387
<i>ESR2 AluI</i>	A vs. G allele	AA vs. GG	AG vs. GG	AA + AG vs. GG	AA vs. AG + GG
Begg's test	0.175	0.174	0.266	1.000	0.711
Egger's test	0.168	0.078	0.551	0.578	0.119

Over the past few decades, a growing body of evidence has implied that estrogens play a vital role on male reproductive capability [5]. The effects of estrogens are mediated by at least two ER isoforms (ER α and ER β), which are expressed in human germ cells of various stages [16, 6]. The importance of ERs in male reproduction has been elucidated by both genetically modified mice without functional ER α and/or ER β [43, 16] and the phenotypes of men with ER α and/or ER β gene mutations [44, 33]. As one form of the most common genetic abnormalities, the SNPs in *ESR1* and *ESR2* and their implications in male infertility have been investigated widely.

Since the first two studies addressing the relationship of *ESR1* and *ESR2* polymorphisms with male infertility were reported [19, 21], a number of similar studies were conducted in different ethnicities with inconclusive results. After pooling all data from seven eligible studies, the results demonstrated that *ESR1 PvuII* variant C allele carriers were significant associated with a decreased male infertility risk (CT vs. TT, OR=0.78, 95 % CI: 0.62–0.98). Further subgroup analyses based on ethnicities demonstrated the differential association of *ESR1 PvuII* polymorphism with a decreased risk in Asian population and an increased risk in Caucasian population. In case of *ESR1 XbaI* polymorphism, the variant G allele was significantly with a lower risk for male infertility in Asian population rather than Caucasian men. With regard to the correlation of *ESR2 RsaI* polymorphism with male infertility, differential even controversial results were presented. Variant A allele was associated with a lower risk in homozygote comparison (AA vs. GG, OR=0.56, 95 % CI: 0.32–0.98) and recessive model (AA vs. AG + GG, OR=0.54, 95 % CI: 0.31–0.93), while an increased risk in heterozygote comparison (AG vs. GG, OR=1.54, 95 % CI: 1.06–2.24). The subsequent subgroup analysis by ethnicities demonstrated the examined SNP decreased spermatogenic failure risk in Asian males (AA vs. GG, OR=0.56, 95 % CI: 0.31–0.98; AA vs.

AG + GG, OR=0.54, 95 % CI: 0.31–0.94) while increased the risk in Caucasian men (A allele vs. G allele, OR=2.35, 95 % CI: 1.14–4.84; AG vs. GG, OR=2.87, 95 % CI: 1.32–6.22 and AA + AG vs. GG, OR=2.65, 95 % CI: 1.24–5.63). The different results in two ethnic groups contributed to the differential results in various comparison models of overall analysis. As for *ESR2 AluI* polymorphism, no significant association was detected in either overall analysis or subgroup analyses by ethnicities and genotyping methods.

The differential or even controversial results about the association between the three SNPs (*PvuII*, *XbaI* and *RsaI*) may be attributed to the following reasons: 1) the inherent genetic difference between Asians and Caucasians, and the similar result was demonstrated in a meta-analysis addressing the association between *ESR1* polymorphisms and endometrial cancer risk [45]; 2) the difference of sample size in two populations was so obvious as the number of Caucasian studies included in the analysis for the four SNPs ranges from one to three; 3) the difference of lifestyles between Asians and Caucasians, as the phytoestrogens intake and exposure to environmental endocrine disruptors (EEDs) differs in two populations, which can help contribute to male infertility to some degree [5, 32, 46]; 4) the polygenic nature of male infertility is yet unclear, neither is the underlying genetic mechanism which means that additional loci might be involved in the development of the spermatogenic phenotype, either within or near the ERs gene or the other core genes involved in estrogenic and estrogen-related pathways [29, 33].

Although the meta-analysis is robust, several limitations should be acknowledged when interpreting the results. First, this study was conducted at the study level without access to more detailed information such as age, family history, and lifestyle (such as phytoestrogen intake and exposure to EEDs during the neonatal life), which may influence the results. Second, the between-study heterogeneity was significantly

observed during the meta-analysis, even the sensitivity analysis confirmed no substantial impact of a single study on the overall results. Third, the number of studies included to assess the correlation of ERs polymorphisms with male infertility in Caucasian population and with TaqMan PCR genotyping method was relatively small. Last but not least, the meta-analysis is retrospective due to the methodological limitations. In order to minimize the bias, we followed the protocol designed before initiating the study, and the process of studies selection, data extraction and analyses was performed by two independent authors, discrepancies were resolved by discussion with a third author. Nevertheless, the results of this meta-analysis should be interpreted with caution.

Conclusion

In summary, this meta-analysis suggested that polymorphisms in ERs (*ESR1* and *ESR2*) may have differential roles in the predisposition to male infertility due to the different ethnic backgrounds. Additionally, further well-designed and unbiased studies with larger sample size, different genotyping methods, and diverse ethnic backgrounds (especially in Caucasians and Africans) should be conducted to verify our findings.

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