REVIEW

Association of Polymorphisms in Estrogen Receptors with Non-obstructive Azoospermia and Severe Secretory Oligozoospermia: A Meta-Analysis

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Purpose: Estrogen receptor (ER) genes play key roles in male and female reproduction. Non-obstructive azoospermia (NOA) and severe secretory oligozoospermia (SOL) are the most severe and complex conditions impacting male fertility. This meta-analysis aimed to study the association between PvuII (rs2234693, 397T>C), XbaI (rs9340799, 351G>A), AluI (1730G>A, rs4986938), and RsaI (1082G>A, rs1256049) polymorphisms and spermatogenic failure.

Materials and Methods: The literature in PubMed, Medline, Embase, Web of Science, Cochrane Library, China Science and Technology Journal Database, WanFang data, and China National Knowledge Infrastructure databases was systematically searched, and a meta-analysis was conducted to investigate the association between polymorphisms in estrogen receptors and spermatogenic failure. According to a set criterion, 10 studies were included for analysis.

Results: The ERα XbaI polymorphism was associated with a decreased risk of NOA. The ERα PvuII polymorphism is not associated with NOA and SOL. The ERβ AluI polymorphism increased the risk of NOA in the Caucasian population. The ERβ RsaI polymorphism was associated with a decreased risk of NOA and SOL in Caucasian males.

Conclusion: The ERα XbaI and ERβ RsaI polymorphisms are associated with the risk of NOA and SOL.

Keywords: estrogen receptor; spermatogenic failure; non-obstructive azoospermia; oligozoospermia; meta-analysis

INTRODUCTION

Infertility is an essential health and social problem, and approximately 40-50% of overall infertility cases involve issues with male fertility.[1,2] The causative factors responsible for male infertility include congenital and acquired factors.[3-5] Non-obstructive azoospermia (NOA) and severe secretory oligozoospermia (SOL, sperm concentration < 5×10^6/mL) affect approximately 1% of the general male population and 10% of men with infertility.[6] Clinically, NOA and SOL are considered to be the most severe conditions associated with male infertility. However, little is known about the cause of these conditions, especially the underlying mechanisms of NOA and SOL. Most etiologies of NOA or SOL remain idiopathic. Presumably, part of these conditions could be explained as being caused by a genetic disorder.[6,7]

Estrogens are considered feminine hormones, but extensive study has shown that estrogens play an essential role in the process of spermatogenesis.[7] Estrogens are bound to either intracellular or membrane estrogen receptors (ERs). ERs play a biological role through genomic or non-genomic signaling pathways. ERs are essential to estrogen signaling. These pathways are involved in the regulation of some urology system diseases, such as renal calcium stone disease and infertility.[9] ERs are of two subtypes: ERα and ERβ. ERα and ERβ are proteins composed of 595 and 530 amino acids, respectively. ERα and ERβ are encoded by two different genes on chromosome 6q25 and 14q23-24, respectively.[10] Both ERα and ERβ gene loci contain several single nucleotide polymorphisms (SNPs).[11] PvuII (rs2234693, 397T>C) and XbaI (rs9340799, 351G>A) are restriction fragment length polymorphisms in intron I separated by 46 bp. These two SNPs have been the most investigated in ERα, whereas two silent G/A SNPs, AluI (1730G>A, rs4986938) and RsaI (1082G>A, rs1256049), have been extensively studied for ERβ.[12]

Some studies have researched the association between ER polymorphisms and male infertility. However, few studies have analyzed ERα and ERβ SNPs in patients with NOA or SOL. Hence, the results of this association remain inconsistent. Therefore, we conducted this meta-analysis to study the association between ER SNPs and NOA or SOL.

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Table 1. The general characters of included studies in the meta-analysis

Author	Year	Country	Race	Polymorphism sites	Genotyping method	Case definition and size(n)	HWE
Aschim	2005	Sweden	Caucasian	ESRβ(RsaI, AluI)	PCR-RFLP	Severe secretory oligozoospermia (10^6)	0.061
Khattri	2009	Indian	Asian	ESRβ(RsaI)	PCR-RFLP	NOA (271)	0.490
Bianco	2011	Brazil	Caucasian	ESRα(XbaI, PvuII),ESRβ(RsaI, AluI)	TaqMan assays	NOA (78), Severe secretory oligozoospermia (10^9)	0.221
Ogata	2012	Japan	Asian	ESRβ(RsaI)	TaqMan assays	NOA and Severe secretory oligozoospermia (125)	0.604
Yufei Ma	2014	China	Asian	ESRβ(RsaI, AluI)	PCR-RFLP	NOA and Severe secretory oligozoospermia (84)	0.056
Bordin	2015	Brazilian	Caucasian	ESRβ(RsaI)	PCR-RFLP	NOA (44) , Severe secretory oligozoospermia(43)	0.892
Tianfu Li	2015	China	Asian	ESRα(XbaI, PvuII),ESRβ(AluI)	PCR-RFLP	NOA(142) ,Severe secretory oligozoospermia(270)	0.615
Vladoiu	2016	Romania	Caucasian	ESRα(XbaI, PvuII)	PCR-RFLP	NOA and Severe secretory oligozoospermia(42)	0.449
Younes	2016	Egypt	Caucasian	ESRβ(RsaI)	PCR-RFLP	NOA (90)	0.842
Mobasseri	2019	Iran	Caucasian	ESRα(PvuII)	PCR-RFLP	NOA (50)	0.062

NOA: Non-obstructive azoospermia; PCR-RFLP: Polymerase chain reaction-restriction fragment length ploymorphism; ESR: estrogen receptor; HWE: p for Hardy-Weinberg equilibrium

MATERIALS AND METHODS

This meta-analysis was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines.[13] This meta-analysis was registered in the International Platform of Registered Systematic Review and Meta-analysis Protocols with registration number INPLASY2022100034.

Literature Search

PubMed, Medline, Embase, Web of Science, Cochrane Library, Scopus, China Science and Technology Journal Database, WanFang data, and China National Knowledge Infrastructure databases were searched up to July 1st, 2022, without language restrictions. The keywords used were: (Male Infertility[MeSH]) AND (Azoospermia[MeSH]) AND (Non-obstructive Azoospermia[MeSH]) AND (Severe oligozoospermia[MeSH]) AND (Spermatogenic Failure[MeSH]) AND (Estrogen Receptor[MeSH]) OR (ER) OR (ESR) OR (ERα) OR (ERβ) OR (PvuII) OR (397T>C) OR (rs2234693) OR (XbaI) OR (351G>A) OR (rs9340799) OR (AluI) OR (1730G>A) OR (rs4986938) OR (RsaI) OR (1082G>A) OR (rs1256049) AND (Polymorphism) OR (SNP).

Selection Criteria

The main inclusion criteria were: (1) the study must have analyzed the correlation between the PvuII, XbaI, AluI, and RsaI polymorphisms and NOA and/or SOL; (2) the inclusion of patients with a diagnosis of NOA and/or SOL according to the World Health Organization 2010 guideline; (3) the study must include normal sperm parameters or fertile males as negative controls; (4) the control group complies with Hardy-Weinberg equilibrium (HWE); (5) the study must have had enough data for genotype frequencies to be able to calculate the odds ratio (OR) and 95% confidence interval (CI); and (6) the study design had to be a case-control study. The main exclusion criteria were: (1) studies providing insufficient data for analysis, including meta-analyses, article reviews, expert opinions, case reports, and studies involving animals or cell lines; (2) the control group did not comply with HWE; (3) studies from which detailed data could not be extracted and contact with the corresponding authors failed; and (4) low-quality studies.

Data Extraction

Two researchers (S.W. and A.Z.) extracted the data independently, and a third researcher (Y.P.) resolved any discrepancies. The following details were considered while extracting the data from the studies: first author, year of publication, country, race, polymorphism sites, genotyping method, case definition and size, HWE in controls, OR, and 95% CI.

Quality Assessment

The Newcastle-Ottawa Quality Assessment Scale (NOS) was applied to assess the quality of the case-control studies.[14] Studies with scores of < 3, 4-6, and > 7 were classified as low, moderate, and high quality, respectively.

Statistical Analysis

We used the odds ratio (OR) and its corresponding 95% CI to assess the association between the PvuII, XbaI, AluI, and RsaI polymorphisms in the ER gene and the risk of NOA and/or severe secretory oligozoospermia. A *P*-value < .05 was considered statistically significant.

Table 2. Newcastle-Ottawa Scale scores for literature quality assessment.

Author	Year	Country	Study design	Selection			Comparability	Exposure			Total scores
				Adequacy of case definition	Representativeness of cases	Selection of controls	Definition of controls	Ascertainment of exposure		Same method of ascertainment	Non-response rate	
Aschim	2005	Sweden	case-control	1	1	1	1	1	1	1	1	8
Khattri	2009	Indian	case-control	1	1	1	1	1	1	1	1	8
Bianco	2011	Brazil	case-control	1	1	1	1	1	1	1	1	8
Ogata	2012	Japan	case-control	1	1	1	1	1	1	1	1	8
Yufei Ma	2014	China	case-control	1	1	1	1	1	1	1	1	8
Bordin	2015	Brazilian	case-control	1	1	1	1	1	1	1	1	8
Tianfu Li	2015	China	case-control	1	1	1	1	1	1	1	1	8
Vladoiu	2016	Romania	case-control	1	1	0	1	1	1	1	1	7
Younes	2016	Egypt	case-control	1	1	1	1	1	1	1	1	8
Mobasseri	2019	Iran	case-control	1	1	0	1	1	1	1	1	7

Table 3. Analysis results of ER α PvuII polymorphism on non-obstructive azoospermia and severe secretory *oligozoospermia*

ERs polymorphisms in NOA and SOL-Zhang et al.

Variables	T vs. C TT vs. CC			TC vs. CC+ TT						TT+TC vs. CC			TT vs. TC		
	P	OR (95%CI)	I^2	*P*	OR (95%CI)	I^2	*P*	OR (95%CI)	I^2	*P*	OR (95%CI)	I^2	*P*	OR (95%CI)	I^2
Overall	0.302	0.80 (0.53-1.22)	82.2%	0.779	0.93 (0.58-1.82)	60.0%	0.213	0.68 (0.37-1.25)	77.2%	0.187	1.17 (0.92-1.49)	4.6%	0.338	0.69 (0.31-1.50)	77.2%
Ethnicity															
Caucasians	0.092	0.66 (0.41-1.07)	70.3%	0.245	0.76 (0.49-1.20)	11.7%	0.080	0.51 (0.24-1.09)	70.4%	0.560	0.88 (0.57-1.35)	0.0%	0.101	0.47 (0.19-1.16)	61.1%
NOA															
Total	0.67	0.93 (0.67-1.24)	49.6%	0.636	1.08 (0.80-1.45)	14.0%	0.255	0.82 (0.59-1.15)	34.3%	0.396	1.15 (0.83-1.58)	0.0%	0.572	0.89 (0.60-1.33)	49.5%
Ethnicity															
Caucasians	0.135	0.81 (0.61-1.07)	0.0%	0.433	0.82 (0.50-1.34)	0.0%	0.095	0.68 (0.44-1.07)	31.9%	0.818	0.94 (0.56-1.58)	0.0%	0.131	0.64 (0.35-1.14)	31.1%
SOL															
Total	0.440	1.14 (0.081-1.60)	66.0%	0.054	1.31 (0.99-1.72)	44.2%	0.271	1.20 (0.87-1.67)	45.6%	0.123	1.26 (094-1.68)	13.3%	0.459	1.29 (0.66-2.56)	61.4%
Ethnicity	-														
Begg's testa	0.052			0.497			0.052			0.497			0.497		

p: p values for effect; a: p values for Begg's test; b: p values for Egger's test.

Heterogeneity was tested using the Chi-square-based Q test and Higgins I^2 statistics. An $I^2 < 50\%$ indicated that no heterogeneity existed, and the fixed-effects model (FEM) was used; otherwise, the random-effects model (REM) was used. We used five genetic models to calculate the pooled OR: (1) allelic model: A vs. a; (2) recessive model: AA vs. Aa + aa; (3) dominant model: AA + Aa vs. aa; (4) heterozygote comparison: Aa vs. aa; and (5) homozygote comparison: AA vs. aa. Data from the meta-analysis were analyzed by STATA 12.0 (STATA Corporation, College Station, TX, USA).

Publication Bias and Sensitivity Analyses

Publication bias was tested using Egger's and Begg's tests. Sensitivity analyses were performed to estimate the stability of the results. HWE in the controls was analyzed using STATA 12.0 (STATA Corporation, College Station, TX, USA) and GraphPad Software (San Diego, CA, USA).

Subgroup and Stratified Analyses

To study the association of polymorphisms in ERs with risks of spermatogenic failure in different ethnicities and degrees of spermatogenic failure, subgroup analyses based on different disease types (NOA and SOL) and different races were conducted.

RESULTS

Study Selection and Characteristics

Figure 1 shows the procedures used for searching the studies. In total, 216 potential studies were identified from the databases. After reading the titles and abstracts, 104 potentially related publications were excluded. After reading the full texts, we included 10 articles in this meta-analysis.[15-24] All 10 studies were case-control studies. Table 1 shows the principal characteristics of the included studies. These studies were of high quality (**Table 2**). Four of the studies reported PvuII polymorphism, three reported XbaI polymorphism, four reported AluI polymorphism, and seven reported RsaI polymorphism associated with NOA and SOL, respectively. Six articles pertained to studies conducted in Caucasian populations and four in Asian populations. Three studies related to NOA and SOL, six to NOA only, and four to SOL only.

Table 4. Analysis results of ER α XbaI polymorphism on non-obstructive azoospermia and severe secretory oligozoospermia

Variables	G vs. A *P*	GG vs. AA+AG OR (95%CI)	I^2	GG+AG vs. AA *P*	OR (95%CI)	I^2	GG vs. AG *P*	OR (95%CI)	I^2	GG vs. AA *P*	OR (95%CI)	I^2
Overall	0.512	1.06 (0.89-1.27)	0.0%	0.858	0.96 (0.64-1.45)	0.0%	0.292	2.20 (0.51-9.59)	96.0%	0.704	0.92 (0.60-1.42)	0.0%
Ethnicity												
Caucasians	0.777	0.96 (0.73-1.26)	0.0%	0.699	0.90 (0.54-1.51)	0.0%	0.346	8.19 (0.10-651.1)	96.5%	0.700	0.90 (0.52-1.55)	0.0%
NOA												
Total	0.926	0.99 (0.76-1.28)	0.0%	0.570	0.83 (0.44-1.58)	0.0%	0.002	0.06 (0.01-0.35)	94.3%	0.515	0.80 (0.41-1.57)	0.0%
Ethnicity	-											
SOL												
Total	0.226	0.88 (0.71,1.09)	0.0%	0.948	0.98 (0.59-1.65)	0.0%	0.899	1.03 (0.60-1.77)	0.0%	0.854	0.95 (0.55-1.64)	0.0%
Ethnicity	-											
Begg's test a	0.602			0.117			0.117			0.117		
Egger's test b	0.962			0.367			0.100			0.183		

Variables	GG vs. AA *P*	OR (95%CI)	I^2
Overall	0.896	0.97 (0.62-1.52)	0.0%
Ethnicity			
Caucasians	0.618	0.86 (0.47-1.56)	0.0%
NOA			
Total	0.001	0.11 (0.03-0.39)	66.1%
Ethnicity			
SOL			
Total	1.000	1.00 (0.49-2.02)	0.0%
Ethnicity			
Begg's test a	0.602		
Egger's test b	0.645		

p: p values for effect; a: p values for Begg's test; b: p values for Egger's test.

Table 5. Analysis results of ER β AluI polymorphism on non-obstructive azoospermia and severe secretory oligozoospermia

Variables	G vs. A	GG vs. AA+AG		GG+AG vs. AA		GG vs. AG	GG vs. AA							
	P	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2
Overall	0.507	1.06 (0.90-1.25)	0.0%	0.556	1.07 (0.86-1.32)	0.0%	0.212	1.27 (0.87-1.85)	0.0%	0.603	1.06 (0.85-1.32)	0.0%	0.596	1.11 (0.75-1.66)	0.0%
Ethnicity															
Caucasians	0.494	1.08 (0.87-1.35)	0.0%	0.639	1.08 (0.79-1.48)	0.0%	0.109	1.43 (0.92-2.21)	0.0%	0.806	1.04 (0.75-1.45)	0.0%	0.430	1.21 (0.75-1.94)	0.0%
Asians	0.820	1.03 (0.80-1.33)	0.0%	0.712	1.05 (0.79-1.40)	17.0%	0.763	0.89 (0.42-1.89)	0.0%	0.632	1.08 (0.80-1.45)	23.6%	0.789	0.90 (0.42-1.92)	0.0%
NOA															
Total	0.386	1.13 (0.85,1.50)	0.0%	0.070	1.43 (0.97-2.12)	0.0%	0.409	0.82 (0.50-1.32)	29.5%	0.165	1.69 (0.81-3.53)	64.0%	0.213	1.50 (0.79-2.86)	0.0%
Ethnicity - SOL															
Total	0.926	1.01 (0.83-1.22)	0.0%	0.531	1.17 (0.72-1.90)	69.3%	0.654	0.92 (0.64-1.32)	0.0%	0.474	1.24 (0.68-2.264)	76.7%	0.320	1.24 (0.81-1.92)	25.2%
Ethnicity															
Caucasians	0.357	1.12 (0.88-1.43)	0.0%	0.332	1.43 (0.70-2.92)	71.8%	0.894	0.97 (0.30-1.44)	0.0%	0.397	1.55 (0.56-4.27)	83.3%	0.104	1.52 (0.92-2.50)	0.0%
Begg's test a	0.602			0.117			0.117			0.117			0.117		
Egger's test b	0.413			0.315			0.019			0.353			0.225		

p: p values for effect; a: p values for Begg's test; b: p values for Egger's test.

Meta-analysis Results

Effects of ERα PvuII polymorphism on NOA and SOL

For NOA and SOL, PvuII polymorphism was reported in four studies[16,18,21,23] (Case = 691, Control = 911). The overall effect of the ERα PvuII polymorphism was not associated with NOA and SOL (**Figure 2A, Table 3**). Subgroup analyses based on race showed that ERα PvuII polymorphism has no association with spermatogenic failure in Caucasians (**Figure 2A, Table 3**). Only one study[18] reported ERα PvuII polymorphism as an increased risk of NOA and SOL in Asian males; however, data were insufficient for the subgroup analyses.

For NOA, PvuII polymorphism was reported in three studies[16,18,23] (Case = 270, Control = 883). The total effect of the ERα PvuII polymorphism had no association with NOA (**Figure 2B, Table 3**). Subgroup analyses based on race showed that ERα PvuII polymorphism was not related to NOA in Caucasian or Asian males (**Table 3**).

For SOL, PvuII polymorphism was reported in two studies[16,18] (Case = 379, Control = 670). The total effect of the ERα PvuII polymorphism had no association with SOL (**Figure 2B, Table 3**). Subgroup analyses based on race showed that ERα PvuII polymorphism has no association with SOL in Caucasian males (**Table 3**). However, one study showed that ERα PvuII polymorphism increased the risk of SOL in Asian males[18]; however, data were insufficient for the subgroup analyses.

Effects of ERα XbaI polymorphism on NOA and SOL

For NOA and SOL, XbaI polymorphism was reported in three studies[16,18,21] (Case = 641, Control = 697). The overall effect of the ERα XbaI polymorphism had no association with NOA and SOL (**Figure 2C, Table**

Table 6. Analysis results of ER β RsaI polymorphism on non-obstructive azoospermia and severe secretory oligozoospermia

Variables		G vs. A	GG vs. AA+AG		GG+AG vs. AA		GG vs. AG	GG vs. AA							
	P	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2	*P*	OR(95%CI)	I^2
Overall	0.563	0.73 (0.25-2.11)	94.2%	0.608	0.77 (0.28-2.09)	90.1%	0.457	2.15 (0.29-10.09)	85.1%	0.368	0.70 (0.31-1.54)	84.0%	0.529	2.43 (0.15-38.55)	91.3%
Ethnicity															
Caucasians	0.010	0.41 (0.21-0.81)	46.7%	0.008	0.42 (0.22-0.80)	37.4%	0.594	0.55 (0.06-4.90)	0.0%	0.009	0.43 (0.22-0.81)	36.2%	0.535	0.50 (0.75-4.46)	0.0%
Asians	0.548	1.65 (0.32-8.39)	97.0%	0.517	1.81 (0.30-10.92)	95.7%	0.236	4.27 (0.36-61.45)	93.2%	0.683	1.34 (0.33-5.38)	92.0%	0.297	7.72 (0.42-359.78)	96.3%
NOA															
Total	0.005	0.55 (0.38-0.83)	40.9%	0.008	0.56 (0.36-0.86)	31.4%	0.300	0.21 (0.01-4.07)	Excluded	0.014	0.58 (0.37-0.89)	17.7%	0.272	0.19 (0.01-3.70)	Excluded
Ethnicity															
Caucasians	0.003	0.39 (0.21-0.73)	43.9%	0.007	0.41 (0.22-0.78)	36.3%	0.300	0.21 (0.01-4.07)	Excluded	0.015	0.45 (0.23-0.86)	26.2%	0.272	0.19 (0.01-3.70)	Excluded
SOL															
Total	0.039	0.56 (0.32-0.97)	19.7%	0.026	0.53 (0.30-0.93)	33.5%	0.740	1.72 (0.07-3.20)	Excluded	0.019	0.50 (0.28-0.89)	42.6%	0.785	1.56 (0.06-3.20)	Excluded
Ethnicity															
Caucasians	0.039	0.56 (0.32-0.97)	19.7%	0.026	0.53 (0.30-0.93)	33.5%	0.740	1.72 (0.07-3.20)	Excluded	0.019	0.50 (0.28-0.89)	42.6%	0.785	1.56 (0.06-3.20)	Excluded
Asians	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Begg's test [a]	0.602			0.602			-			0.602			-		
Egger's test [b]	0.739			0.799			-			0.778			-		

Excluded: some study was excluded because no patient with gene type of AA; p: p values for effect; a: p values for Begg's test; b: p values for Egger's test.

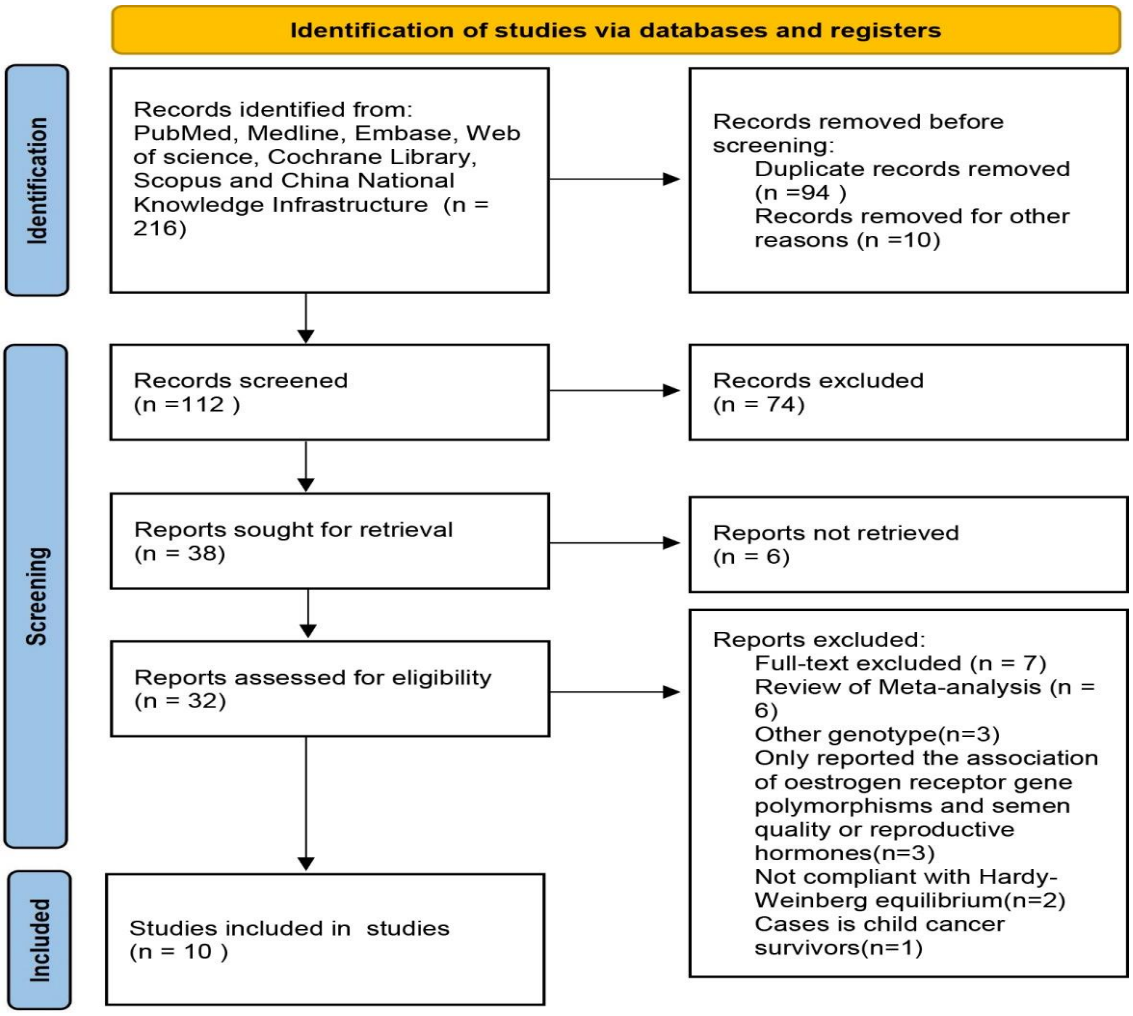

Figure 1. The article selection process.

4). ERα XbaI polymorphism was not associated with spermatogenic failure in Caucasian males **(Figure 2C, Table 4**). Only one study showed that ERα XbaI polymorphism significantly decreased the risk of NOA and SOL in Asian males[18]; however, data were insufficient for the subgroup analyses.

For NOA, XbaI polymorphism was reported in two studies[16,21] (Case = 220, Control = 669). The total effect of the ERα XbaI polymorphism was a decreased risk of NOA (GG+AG vs. AA, OR = 0.06, 95% CI: 0.01 to 0.35, *P* = .002; GG vs. AA, OR = 0.11, 95% CI: 0.03 to 0.39, *P* = .001) (**Figure 2D, Table 4**). However, the studies of Caucasian and Asian populations were from single research articles, respectively(16,18), and data were insufficient for subgroup analyses.

For SOL, ERα XbaI polymorphism was reported in two studies(16,18) (Case=379, Control=670). The total effect of the ERα XbaI polymorphism had no association with SOL (**Figure 2D, Table 4**). Subgroup analyses based on race showed that ERα XbaI polymorphism was not associated with SOL in Caucasian or Asian males (**Table 4**).

Effects of ERβ AluI polymorphism on NOA and SOL

For NOA and SOL, ERβ AluI polymorphism was reported in four studies[14,16,18,19] (Case = 788, Control = 958). The overall effect of the ERβ AluI polymorphism had no association with NOA and SOL (**Figure 2E, Table 5**). Subgroup analyses based on race showed that ERβ AluI polymorphism was not associated with spermatogenic failure in Caucasian or Asian males (**Figure 2E, Table 5**).

For NOA, ERβ AluI polymorphism was reported in two studies[16,18] (Case = 219, Control = 669). The total effect of the ERβ AluI polymorphism had no association with NOA (**Figure 2F, Table 5**). Subgroup analyses based on race showed that ERβ AluI polymorphism increased the risk of NOA in the Caucasian population (GG vs. AG, OR=2.61, 95% CI: 1.23 to 5.51, *P* = .012) (**Table 5**). However, only one study[17] reported that ERβ AluI polymorphism had no association with NOA in Asian males, and data were insufficient for subgroup analyses.

For SOL, ERβ AluI polymorphism was reported in three studies[14,16,18] (Case = 485, Control = 855). The total effect of the ERβ AluI polymorphism did not increase the risk of SOL (**Figure 2F, Table 5**). Subgroup analyses based on race showed that ERβ AluI polymorphism did not increase the risk of SOL in Caucasian or Asian males (**Table 5**).

Effects of ERβ RsaI polymorphism on NOA and SOL

For NOA and SOL, ERβ RsaI polymorphism was re-

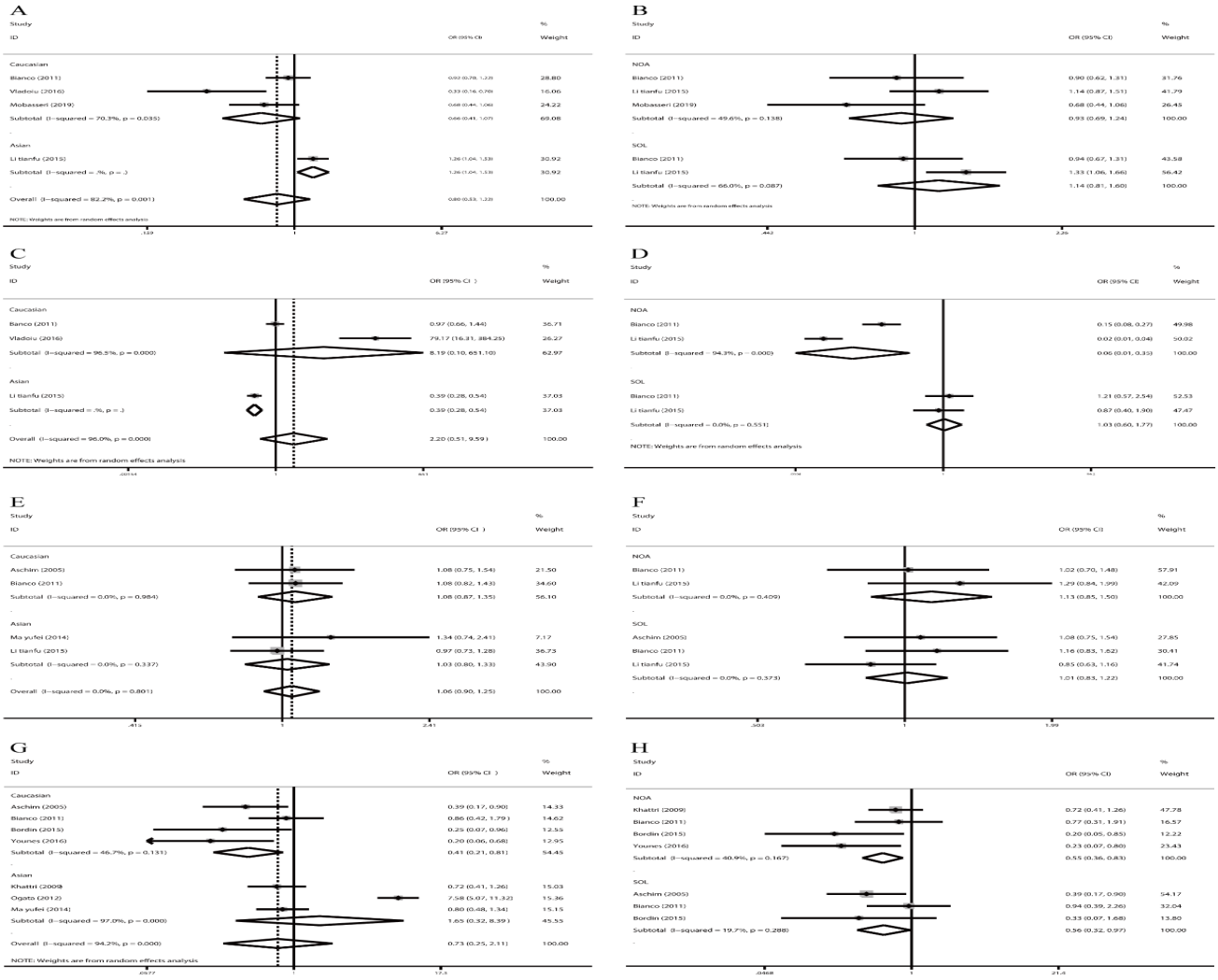

Figure 2. Meta-analysis of effects of ERs polymorphism on non-obstructive azoospermia and severe secretory oligozoospermia.
A: Overall effects of ER α PvuII polymorphism on NOA and SOL (T vs. C); B: Effects of ER α PvuII on NOA or SOL (T vs. C); C: Overall effects of ER α XbaI polymorphism on NOA and SOL (GG+AG vs. AA); D: Effects of ER α XbaI polymorphism on NOA or SOL (GG+AG vs. AA); E: Overall effects of ER β AluI polymorphism on NOA and SOL (G vs. A); F: Effects of ER β AluI polymorphism on NOA or SOL (G vs. A); G: Overall effects of ER β RsaI polymorphism on NOA and SOL (G vs. A); H: Effects of ER β RsaI polymorphism on NOA or SOL (G vs. A)

ported in seven studies[14-17,19,20] (Case = 950, Control = 1061). The overall effect of the ERβ RsaI polymorphism had no association with spermatogenic failure (**Figure 2G, Table 6**). Subgroup analyses based on race showed that ERβ RsaI polymorphism was associated with a decreased risk of NOA and SOL in Caucasian males (G vs. A, OR=0.41, 95% CI: 0.21 to 0.81, *P* = .010; GG vs. AA + AG, OR=0.42, 95% CI: 0.22 to 0.80, *P* = .008; GG vs. AG, OR=0.43, 95% CI: 0.22 to 0.81, *P* = .009)(**Figure 2G, Table 6**). ERβ RsaI polymorphism did not increase the risk of NOA and SOL in Asian males (**Figure 2G, Table 6**).

For NOA, ERβ RsaI polymorphism was reported in four studies[15,16,20,22] (Case=483, Control=654). The total effect of the ERβ RsaI polymorphism was associated with a decreased risk of NOA (G vs. A, OR=0.55, 95% CI: 0.38 to 0.83, *P* = .005; GG vs. AA + AG, OR=0.56, 95% CI: 0.36 to 0.86, *P* = .008; GG vs. AG, OR=0.58, 95% CI: 0.37 to 0.89, *P* = .014) (**Figure 2H, Table 6**). Subgroup analyses based on race showed that ERβ RsaI polymorphism was associated with a decreased risk of NOA in Caucasian males (G vs. A, OR=0.39, 95% CI: 0.21 to 0.73, *P* = .003; GG vs. AA + AG, OR=0.41, 95% CI: 0.22 to 0.78, *P* = .007; GG vs. AG, OR=0.45, 95% CI: 0.23 to 0.86, *P* = .015) (**Table 6**). ERβ RsaI polymorphism was not associated with NOA in Asian males (**Table 6**).

For SOL, ERβ RsaI polymorphism was reported in three studies[14,16,20] (Case=258, Control=528), all of which involved Caucasian populations. ERβ RsaI polymorphism was associated with a decreased risk of SOL in the total effect or in Caucasian males (G vs. A, OR=0.56, 95% CI: 0.32 to 0.97, *P* = .039; GG vs. AA + AG, OR=0.53, 95% CI: 0.30 to 0.93, *P* = .026; GG vs. AG, OR=0.50, 95% CI: 0.28 to 0.89, *P* = .019) (**Figure 2H, Table 6**).

Publication Bias

No evidence of publication bias was found in this study based on Begg's and Egger's tests(**Tables 3,4,5,6**).

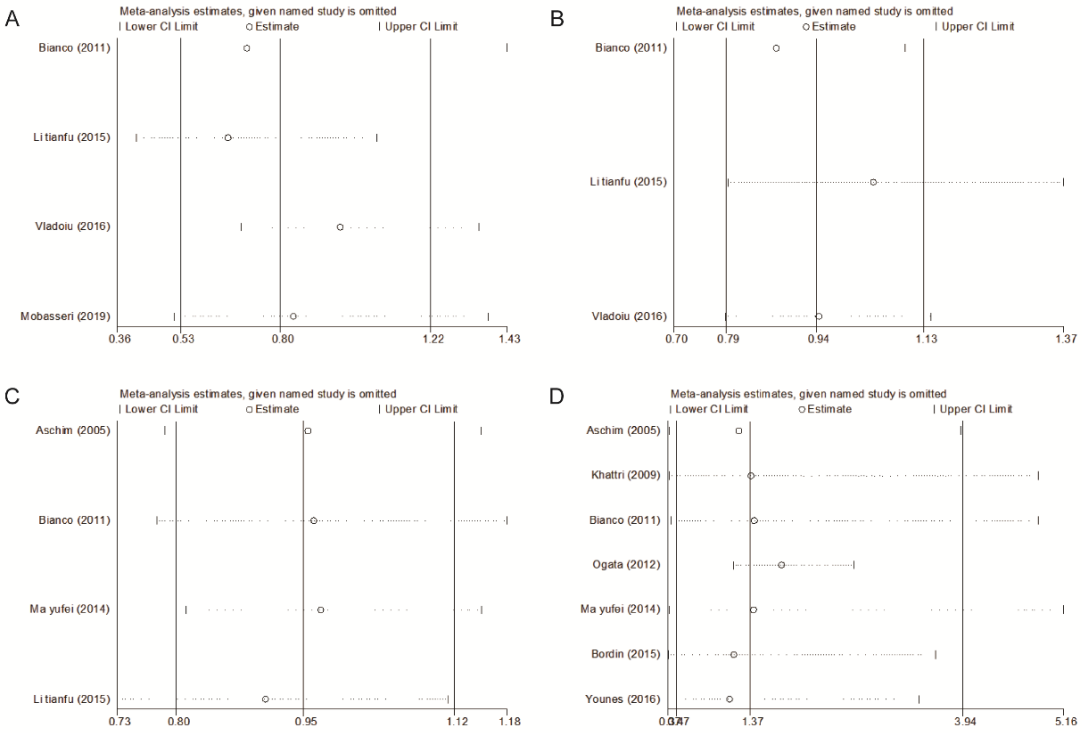

Figure 3. Sensitivity analyses of spermatogenic failure.
A: Sensitivity analyses of T vs. C for ER α PvuII; B: Sensitivity analyses of G vs. A for ER α XbaI.C: Sensitivity analyses of G vs. A for ER β AluI. D: Sensitivity analyses of G vs. A for ER β RsaI.

Sensitivity Analyses

One-way sensitivity analyses were performed on PvuII, XbaI, AluI, and RsaI polymorphisms in five genetic models. The results indicated that these studies were stable (**Figure 3**).

DISCUSSION

NOA and SOL are complex conditions of male infertility in which genetic and environmental factors exert a combined effect. The etiology of NOA or SOL remains primarily unknown.[25] Approximately 20-25% of male patients with infertility experience low sperm quality due to genetic factors.[25,26] ER is expressed in the testis, epididymis, vas deferens, and prostate in males, and has been detected in spermatocytes, spermatids, spermatozoa, Sertoli cells, and Leydig cells.[10] However, mechanisms underlying the association between ERs and spermatogenesis are still unclear. Some studies have indicated that germ cell viability is dependent on the ERα signaling pathway. ERβ-mediated 17β-estradiol actions may affect Sertoli cell proliferation and suppress differentiation.[27] Some studies have researched the association of SNPs in ER genes with the risk of NOA or SOL.[17,18,24] However, the results of this association are yet unclear.

The PvuII and XbaI polymorphisms occur due to the transition of C to T and A to G, respectively. The polymorphisms of PvuII and XbaI both occur in intron 1 of the ERα gene located on chromosome 6q25,[10] and the two SNPs are separated by only 46 bp and are in strong linkage disequilibrium. The RsaI and AluI polymorphisms comprise the exchange of G with A; both are silent polymorphisms in exon 5 of the ERβ gene, on chromosome 14q23-24.[12] ERα and ERβ SNPs are associated with many diseases, such as infertility,(28) premature ovarian failure,[29] osteoporosis,(30) and tibial tendon dysfunction.[31] However, the association of ERα and ERβ SNPs in patients with NOA or SOL is still unclear. In our meta-analysis, we studied the association between the ER SNPs and NOA and/or SOL, including four SNPs (PvuII, XbaI, AluI, and RsaI).

Our results show that the overall effect of the ERα PvuII polymorphism was not associated with NOA and SOL. Meanwhile, the overall effect of the ERα XbaI polymorphism was not associated with NOA and SOL either. However, the incidence of NOA and/or SOL is lower than other diseases with semen abnormalities. Studies of the association between NOA and/or SOL and SNPs of ERα were limited. A study by Kukuvitis et al. found that ERα SNPs were associated with idiopathic azoospermia or severe oligozoospermia.[31] Safarinejad et al. showed that ERα PvuII TT and XbaI AA genotypes were risk factors for the decrease of sperm motility, sperm density, and normal morphology.[32] Zalata et al. found that ERα SNPs were associated with sperm acrosin activity in patients with infertility.[12] Bianco et al. found no association between ERα SNPs and concluded that they were not relevant to idiopathic infertility.[16] Mahato et al. found that spermatogenic cells in male mice do not require ERα for development or function.[33] Thus, previous studies support our results that ERα PvuII and XbaI polymorphisms are not associated with NOA and SOL. However, not enough studies have been conducted to get accurate results for subgroup analysis pertaining to race. Only a single study has shown that the ERα XbaI polymorphism was

associated with a decreased risk of NOA and SOL in the dominant model for Asian males.

Nevertheless, the results of the association between ERβ SNPs and NOA or SOL are still controversial. Ogata et al. conducted a study and found that the ERβ specific SNPs raise the risk of spermatogenic failure in the Japanese population.[17] However, Bianco et al. found that ERβ SNPs were not relevant to NOA and SOL in the Brazilian population.[16] A meta-analysis by Cai Y et al. found that ERβ GA and GG of AluI SNPs were resistant to deteriorated sperm quality.[34] In our meta-analysis, the overall effect of the ERβ AluI polymorphism was not associated with NOA and SOL, and not enough data were available to obtain accurate results of subgroup analysis for race. We found that the variant G allele of ERβ RsaI polymorphism is a protective factor for NOA and SOL in the Caucasian population. Subgroup analyses based on the type of disease and ethnicity show that the G allele of ERβ RsaI polymorphism is a protective factor in both NOA and SOL for the Caucasian population. The ERβ RsaI polymorphism is not associated with NOA and SOL in the Asian population.

In this meta-analysis, we included only high-quality studies, and we did not find evidence of publication bias. The sensitivity analyses showed that the results of our study are stable. This meta-analysis focused on the association of polymorphisms in ERs with NOA and SOL, which could help clinicians, particularly andrologists, to understand the mechanism of idiopathic NOA and SOL. Our study reminds clinicians to pay more attention to potential risk factors such as polymorphisms in estrogen receptors.

The limitation of this study was that the sample size of some studies was not large enough to provide an accurate subgroup analysis. Another limitation was that the included studies excluded men with known genetic causes of infertility and those with genitourinary malformations; however, not all studies properly differentiated the types of azoospermia and oligozoospermia. More research is required to confirm the association of polymorphisms in ERs with NOA and SOL and to explore the related mechanisms.

CONCLUSIONS

The ERα XbaI polymorphism was associated with a decreased risk of NOA. The ERα PvuII polymorphism is not associated with NOA and SOL. The ERβ AluI polymorphism increased the risk of NOA in the Caucasian population. The ERβ RsaI polymorphism was associated with a decreased risk of NOA and SOL in Caucasian males. More research is required to confirm the association of ER polymorphisms with NOA or SOL and to explore the related mechanisms.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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