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GENETICS

SNPs in ERCC1, ERCC2, and XRCC1 genes of the DNA repair pathway and risk of male infertility in the Asian populations: association study, meta-analysis, and trial sequential analysis

Vertika Singh[1] · Sandeep Kumar Bansal[2] · D. V. S. Sudhakar[3] · Neelabh[4] · Arijit Chakraborty[1] · Sameer Trivedi[5] · Gopal Gupta[2] · Kumarasamy Thangaraj[3] · Singh Rajender[2] · Kiran Singh[1]

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Abstract

Purpose We investigated if substitutions in the *ERCC1*, *ERCC2*, and *XRCC1* genes of the DNA repair pathway correlate with non-obstructive azoospermia and male infertility.

Methods A total of 548 azoospermic infertile males and 410 fertile controls were genotyped for *XRCC1* 399A > G, 280G > A, and *ERCC1* C > A 3′ UTR and 541 azoospermic infertile males and 416 fertile controls were genotyped for *ERCC2* 751A > C using iPLEX Gold Assay. Meta-analyses were performed on *XRCC1* 399A > G (1022 cases and 1004 controls), *ERCC1* C > A 3′ UTR (879 cases and 1059 controls), and *ERCC2* 751A > C (914 cases and 850 controls) polymorphisms to quantitatively estimate the significance of the association between these polymorphisms and the risk of infertility.

Results Statistically significant association between *ERCC2* 751A > C SNP and male infertility was found using the codominant model ($p = 0.03$). Results of meta-analysis suggested a lack of correlation with male infertility risk, which could be due to pooling of studies from different ethnic populations. Due to limited the number of studies, a stratified analysis for different ethnic groups could not be performed.

Conclusion (s) In conclusion, AA genotype of 751A > C SNP in *ERCC2* correlated with a higher risk of male infertility and may contribute to an increased risk of azoospermia and male infertility in Indian men.

Keywords DNA repair · Polymorphisms · Infertility · Spermatogenesis

Introduction

Infertility affects 10–15% of couples worldwide, and the cause is male borne in around 50% of them [1]. Though significant progress has been made in the last few years in understanding infertility, more than half of the causes remain unknown or idiopathic [2]. Azoospermia, characterized by the absence of spermatozoa in the ejaculate is the most common, but poorly understood cause of male infertility [3]. Exposure to endogenous and exogenous mutagens can give rise to DNA damage in highly proliferative germ cells [4]. Germ cell DNA damage has been found to be one of the major causes of complete spermatogenic failure and male infertility [5, 6]. To prevent the propagation of heritable genetic mutations in the germ line and to safeguard the genomic integrity and the quality of developing germ cells, a complex DNA repair mechanism is in place [7]. Among various DNA repair systems, the base excision repair (BER) and nucleotide excision repair (NER) are two crucial mechanisms that repair localized small lesions and

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✉ Singh Rajender
rajender_singh@cdri.res.in

✉ Kiran Singh
singhk4@rediffmail.com; skiran@bhu.ac.in

[1] Department of Molecular & Human Genetics, Banaras Hindu University, Varanasi 221005, India

[2] Division of Endocrinology, Central Drug Research Institute, Lucknow, UP, India

[3] CSIR-Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India

[4] Department of Zoology (MMV), Banaras Hindu University, Varanasi 221005, India

[5] Department of Urology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

bulky DNA damages, respectively [7]. Both of these pathways are critical for repairing DNA damage in testicular cells [8, 9]. Variations in the DNA repair capacity are genetically determined and DNA polymorphisms. It may result in subtle structural alterations in the DNA repair proteins resulting in modulation of sperm production [10].

ERCC2 is a component of the NER pathway that is an integral member of the basal transcription factor BTF2/TFIIH [11]. Reports on the cDNA microarray indicated that the expression of the *ERCC2* gene was significantly downregulated in azoospermia testes in comparison to the normal testes [11]. *ERCC1* is another essential component of the NER pathway, where it acts in a complex with XPF to repair the DNA lesions [12]. *Ercc1*-deficient testis displays an increased level of DNA strand breaks, oxidative DNA damage, and apoptosis in the male germ cells [12]. *XRCC1* was the first in a series of cloned DNA repair genes from the BER pathway that acts as a "scaffold" in drawing different components to the site of DNA damage, promoting the efficiency of the BER pathway [13]. Interestingly, its expression is significantly high in the testis, particularly in pachytene spermatocytes and round spermatids [14].

Owing to the critical role in spermatogenesis, variations in the *ERCC2*, *ERCC1*, and *XRCC1* genes might compromise sperm production, resulting in male infertility. Several single-nucleotide polymorphisms (SNPs) in these genes were shown to alter DNA repair efficiency leading to various disease outcomes [15]. However, the information available on the polymorphisms in the *ERCC2*, *ERCC1*, and *XRCC1* genes in male infertility were inconsistent and variable. In the present case-control study, we have studied the correlation of *ERCC2* rs13181 (A > C, p.Lys751Gln; Exon 23), *ERCC1* rs3212986 (C > A, 3′ UTR), and *XRCC1* rs25487 (A > G, p.Gln399Arg; Exon 10) and rs25489 (G > A, p.Arg280His; Exon 9) polymorphisms with azoospermia. Studies have corroborated that individuals with Lys/Lys codon 751 genotype of *ERCC2* rs13181 SNP have reduced DNA repair proficiency than those having a 751Gln allele [16]. Also, the expression of the *ERCC2* gene is found to be significantly downregulated in azoospermic testis compared to the normal testis [17]. Similarly, the results of *ERCC1* rs3212986 minor A allele is associated with reduced DNA repair proficiency and poor survival of tumors cells [18]. It has been shown that A allele of *XRCC1* rs25487 SNP results in a higher number of chromosomal aberrations in cancer patients [19]. Furthermore, *XRCC1* rs25489 SNP (R280H) results in the formation of a variant protein which is defective in its efficient localization to a damaged site in the chromosome, resulting in reduced cellular BER/SSBR [20].

ERCC2 751A > C, *ERCC1* C > A, and *XRCC1* 399A > G polymorphisms have been previously studied in male infertility [4, 7, 21–23]; however, the results so far have been inconsistent. Hence, we also performed meta-analyses on all eligible case-control studies to quantitatively estimate the significance of the association between these polymorphisms and the risk of male infertility. Due to lack of the availability of literature for the association of *XRCC1* 280 G > A SNP and male infertility risk, we could not perform meta-analysis for this SNP.

Methods

Ethics statement

The study was approved by the Institutional Human Ethics Committee of the Institute of Science, Banaras Hindu University, Varanasi (Approval letter No. Dean/2011-12/119). The experiments were performed as per standard guidelines accepted for research on human samples. Written informed consent was obtained from each enrolled participant after providing detailed information about the purpose of sample collection.

Study group

All the subjects were of Indo-European ethnicity and had infertility persisting for longer than 1 year. All the subjects underwent detailed medical and physical examinations before sample collection. Patients with gross dysmorphic abnormalities, endocrine defects (hypogonadism), acquired and congenital structural defects of the urogenital system, such as those with cystic fibrosis and Young's syndrome, history of pelvic/spinal injuries, cytogenetic aberrations, karyotype abnormalities, and AZF microdeletions were excluded. Patients presenting with the history of genital tract obstruction or/and dysfunction (varicocele, obstructive azoospermia) were also excluded. Patients having extreme alcohol consumption, smoking, drug abuse, and those having been exposed to chemotherapeutics or radiation were also excluded. The possibility of involvement of female factors was excluded before the enrolment of male patients.

Collection of semen samples was performed as per the WHO criteria for semen collection and analysis [24]. Semen analyses were carried out thrice after 3 to 4 days of sexual abstinence to ascertain infertility status of the patients. The diagnosis of azoospermia was confirmed after semen centrifugation followed by pellet analysis that was performed at least twice to ascertain azoospermia phenotype. The controls belonged to the same age group (20–45 years) and had the same ethnicity as the patients. A confirmation of paternity in the last 2 years was considered as a proof of their fertility. A total of 548 infertile cases and 410 fertile controls were utilized for genotyping of *XRCC1* 399A > G, 280G > A, and *ERCC1* C > A 3′ UTR and 541 infertile cases and 416 fertile controls were used for genotyping of *ERCC2* 751A > C.

DNA preparation, plus-minus polymerase chain reaction for Y chromosome deletion mapping and genotyping

Peripheral blood was used for genomic DNA isolation using DNA/RNA extraction kits (Illumina, San Diego, California, USA) following the manufacturer's protocol. DNA quality and quantity was estimated using a NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). Y chromosome microdeletions were analyzed using 11 sets of sequence-tagged site (STS) markers (STSs: sY83, sY84, sY69, sY117, sY152, sY255, sY254, sY157, sY158, sY159, sY160) on both sides of the euchromatic region of the Y chromosome from centromere to interval 7. None of the patients showed deletions of the Y chromosome (Supplementary Fig. 1). For case-control association study, all the SNPs were genotyped using iPLEX Gold Assay for SNP Genotyping.

Meta-analysis of *ERCC2:* rs13181 (A> C, p.Lys751Gln), *ERCC1:* rs3212986 (C > A, 3′ UTR) and (*XRCC1*): rs25487 (A > G, p.Gln399Arg

Literature search

Relevant studies were selected by searching "Google Scholar (scholar.google.co.in)" "ScienceDirect (www.sciencedirect.com)" and "PubMed (www.pubmed.com)" databases up to the 27th of March 2018 as the publication date, using the keywords: "ERCC2," "ERCC1," "XRCC1," "polymorphism," and "male infertility" in various combinations. All the studies were scrutinized for their references to identify other relevant studies. Only the studies published in the English language were considered for further analysis. We did not stipulate a minimum sample size as the standard for inclusion of a study in the analysis. To avoid instances of "double-counting" of the data and overstating the risk estimate, we included only the articles with the largest and most complete data for duplicate studies. To prevent errors in the pooled analysis, the data extraction was performed by VS and SKB, independently.

Data extraction

The data against the following variables were obtained from each study; first author's name, year of publication, ethnicity of subjects, the source of the samples, and genotypes of cases and controls.

Inclusion and exclusion criteria

The hits achieved through literature search were subjected to the following inclusion/exclusion criteria to select the studies for pooled analysis; the inclusion criteria comprised of the following: (i) The studies aimed at analyzing the correlation of *ERCC2* 751A > C, *ERCC1* C > A, 3′ UTR, and *XRCC1*, 399A > G substitutions with male infertility risk. (ii) Each study was an independent case-control study. (iii) The purpose of all the studies and statistical approaches were similar. (iv) The provided information was sufficient to calculate the odds ratio. (v) SNP genotyping was performed using standard genotyping techniques. (vi) Patients within the study were recruited as per the standard diagnostic parameters.

The exclusion criteria included: (i) The study was not a case-control study. (ii) The study did not intend to look for correlation of *ERCC2* 751A > C, *ERCC1* C > A, 3′ UTR, and *XRCC1*, 399A > G substitutions with male infertility risk. (iii) The study had reviewed the literature and not presented new data. (iv) The raw data were not available in the article and the authors did not respond to three requests by e-mail. (v) The study had been extended to include more samples at a later stage.

Statistical analysis

In the meta-analysis of overall samples, the effectiveness of association between *ERCC2* 751A > C, *ERCC1* C > A 3′ UTR, and *XRCC1* 399A > G polymorphisms and male infertility risk was evaluated by pooled odds ratio (OR) with the corresponding 95% confidence interval (CI). The pooled OR and the corresponding 95% CIs was computed using the fixed effects model (the Mantel-Haenszel method) in the absence of heterogeneity among individual studies; otherwise, a random effects models (the Der Simonian and Laird method) was preferred [25, 26]. As meta-analysis pools data across various studies conducted by different people around the world, we had a priori preference to use the random effects model, which is more stringent and less likely to favor an odd observation, unless there is a real effect. The heterogeneity between individual studies was tested using chi-square-based Q test and p values > 0.05 suggested a lack of heterogeneity across the studies. Calculations of OR and 95% CI were performed using different genetic models: dominant, codominant, and recessive. Bonferroni's correction for multiple testing was not contemplated given a limited number of tests that were pre-hypothesized. Genotype data of the cases and control groups were analyzed for fitness in the Hardy-Weinberg Equilibrium (HWE). Publication bias was evaluated using Egger's linear regression test, followed by visual assessment of the funnel plot. All statistical tests were performed using the Comprehensive Meta Analysis software (version 2).

Sensitivity analysis

As all the studies were not performed using equally stringent protocols, some of them may bias the results in the pooled analysis. A sensitivity analysis was hence carried out to identify the studies that could have significantly

biased the overall inference. The control data from each study was analyzed for fitness in the Hardy-Weinberg equilibrium.

Trial sequential analysis

A meta-analysis might be subject to systematic (bias) or random errors (play of chance) due to repeated significance testing, dispersed data, and potential publication bias [27]. Bias from trials with publication bias, small trial bias, and low methodological quality, may result in false *p* value. Therefore, we used trial sequential analysis (TSA) to overcome these limitations, to detect false positive or negative errors, and to calculate the required information size (RIS) for reliability of meta-analysis [28]. Previous studies have suggested that the outcomes of TSA are more reliable than those of the traditional meta-analysis [29, 30]. As we could retrieve at least three previously published studies for *XRCC1* 399G > A polymorphism, we performed TSA to evaluate the potential publication bias. RIS was calculated considering an overall type-I error of 5% and type-II error of 20%. A two-sided graph is plotted by TSA, where red straight lines are indicative of significance boundaries of the conventional meta-analysis; the blue line shows cumulative *Z*-score, and the red lines sloping inwards represent trial sequential monitoring boundaries with adjusted *p* values.

Results

Case-control study

SNP genotyping for all infertile and fertile control individuals was undertaken using iPLEX Gold Assay. All the SNPs except *ERCC2* (rs13181) fitted well in the Hardy-Weinberg equilibrium (HWE) in the control population (*p* value > 0.05). This indicates the importance of additional testing of this SNP in other populations. Upon generation of data from different populations, a meta-analysis on this SNP would clear if it has a subtle or population-specific role in infertility. Both alleles in the case of A > C homozygous substitution (rs13181) in the *ERCC2* gene were observed in comparable frequencies in the study population. Comparison using dominant and recessive models showed no significant difference between cases and controls, but comparison in the codominant model showed a significant association of this substitution with male infertility ($p = 0.03$). AA genotype correlated with a higher risk of male infertility in comparison to CC. However, no association was found at the allelic level (Table 1).

For C > A substitution (rs3212986) in *ERCC1*, the frequencies of both the heterozygous and the homozygous mutant genotypes were lesser in the infertile group (CA, 44.53%; AA, 8.21%) in comparison to fertile controls (CA, 46.34%; AA, 11.22%), though the difference was not statistically significant (11 vs 12, $P = 0.28$; 11 vs 22, $P = 0.07$) (Table 1). For A > G substitution (rs25487) and *XRCC1* G > A substitution (rs25489), no significant difference in frequencies of allele or genotype was found between infertile group and fertile controls (Table 1).

Meta-analysis

The associations of rs13181, rs3212986, and rs25487 polymorphisms with male infertility risk have been previously evaluated across different populations [4, 7, 21–23]. Hence, we performed meta-analyses on data pooled from all published studies to evaluate the association between these polymorphisms and male infertility.

Eligible studies

ERCC2: rs13181 (A> C, p.Lys751Gln)

Literature survey identified four studies. One of the studies by Ji et al. was excluded as they aimed to study the joint effects of *ERCC2* polymorphisms and polycyclic aromatic hydrocarbon exposure on male infertility [21]. Another study by Liu et al. was excluded as the full text of the article was not available [23]. Hence, a total of three case-control studies (914 cases and 850 controls), including the present study following a strict exclusion-inclusion criterion were included in the meta-analysis (Fig. 1).

ERCC1: rs3212986 (C > A, 3′ UTR)

Three studies were identified through literature search. One of the studies by Ji et al. was excluded as they aimed to study the joint effects of *ERCC1* polymorphisms and polycyclic aromatic hydrocarbon exposure on male infertility [21]. Hence, a total of three case-control studies (879 cases and 1059 controls), including the present study following a strict exclusion-inclusion criterion were included in the meta-analysis (Fig. 1).

XRCC1: rs25487 (A > G, p.Gln399Arg)

Literature search identified six studies. Three studies were excluded as they were not relevant to the aim of this study (association of *XRCC1* 399A > G with male infertility). One of the studies by Ji et al. was excluded as they aimed to study the joint effects of *XRCC1* polymorphisms and polycyclic aromatic hydrocarbon exposure on male infertility [21]. Similarly, another study by Tsuchiya et al. was excluded as it focused on investigating the association of

Table 1 Genotype distributions for *ERCC2*, *ERCC1*, and *ERCC2* polymorphisms in cases and controls

SNP ID	Model	Genotype	Control	Case	OR (95% CI)	*p* value
ERCC2 rs13181	Codominant	C/C	58 (13.94)	54 (10.36)	1	
		C/A	185 (44.47)	232 (44.53)	1.35(0.88–2.04)	0.16
		A/A	173 (41.59)	255 (45.11)	1.59 (1.04–2.42)	0.03*
	Dominant	C/C	58 (13.94)	54 (10.36)	1	
		C/A-A/A	358 (86.06)	487 (89.64)	1.46 (0.98–2.18)	0.05
	Recessive	C/C-C/A	243 (58.41)	286 (54.89)	1	
		A/A	173 (41.59)	255 (45.11)	1.25 (0.97–1.61)	0.08
ERCC1 rs3212986	Codominant	C/C	174 (42.44)	259 (47.26)	1	
		C/A	190 (46.34)	244 (44.53)	0.86 (0.66–1.13)	0.28
		A/A	46 (11.22)	45 (8.21)	0.65 (0.41–1.08)	0.07
	Dominant	C/C	174 (42.44)	259 (47.26)	1	
		C/A-A/A	236 (57.56)	289 (52.74)	0.82 (0.64–1.06)	0.14
	Recessive	C/C-C/A	364 (88.78)	503 (91.79)	1	
		A/A	46 (11.22)	45 (8.21)	1.70 (0.45–1.09)	0.12
XRCC1 rs25487	Codominant	A/A	175 (42.68)	260 (47.45)	1	
		A/G	195 (47.56)	234 (42.7)	0.80 (0.61–1.05)	0.12
		G/G	40 (9.76)	54 (9.85)	0.90 (0.57–1.42)	0.67
	Dominant	A/A	175 (42.68)	260 (47.45)	1	
		A/G-G/G	235 (57.32)	288 (52.55)	0.82 (0.63–1.06)	0.14
	Recessive	A/A-A/G	370 (90.24)	494 (90.15)	1	
		G/G	40 (9.76)	54 (9.85)	1.01 (0.65–1.55)	0.95
XRCC1 rs25489	Codominant	G/G	313 (76.34)	416 (75.91)	1	
		G/A	92 (22.44)	124 (22.63)	1.01 (0.74–1.37)	0.92
		A/A	5 (1.22)	8 (1.46)	1.20 (0.39–3.63)	0.75
	Dominant	G/G	313 (76.34)	416 (75.91)	1	
		G/A-A/A	97 (23.66)	132 (24.09)	1.02 (0.75–1.38)	0.87
	Recessive	G/G-G/A	405 (98.78)	540 (98.54)	1	
		A/A	5 (1.22)	8 (1.46)	1.19 (0.39–3.61)	0.75

* Statistically significant

XRCC1 gene polymorphisms with the susceptibility to testicular germ cell tumors, rather than male infertility [31]. Furthermore, another study by Mirsane et al. was excluded as it investigated the relationship between *XRCC1* polymorphism and alcohol consumption as a risk of male infertility [32]. Hence, a total of four case-control studies (1022 cases and 1004 controls), including the present study following a strict exclusion-inclusion criterion were included in the meta-analysis (Fig. 1).

All the cases involved in these studies were pathologically confirmed and age-matched controls were recruited from healthy populations. The main characteristics of these studies are depicted in the Supplementary data 1.

Pooled analysis

As the studies pooled in this analysis were conducted in different laboratories on various populations, internal heterogeneity was anticipated. We observed both, a significant and non-significant heterogeneity across different analyses; therefore, we have presented results of both fixed effects and random effects models of analysis Table 2. Further, we have implemented analyses using all genetic models to thoroughly dissect the association between the polymorphisms and male infertility risk. None of the genetic models could conclude an association of these polymorphisms and male infertility risk ($P > 0.05$) (Fig. 2).

Sensitivity analysis

All the studies complied with the Hardy-Weinberg equilibrium. None of the studies was found to be sensitive enough to strongly bias the overall conclusion of this meta-analysis.

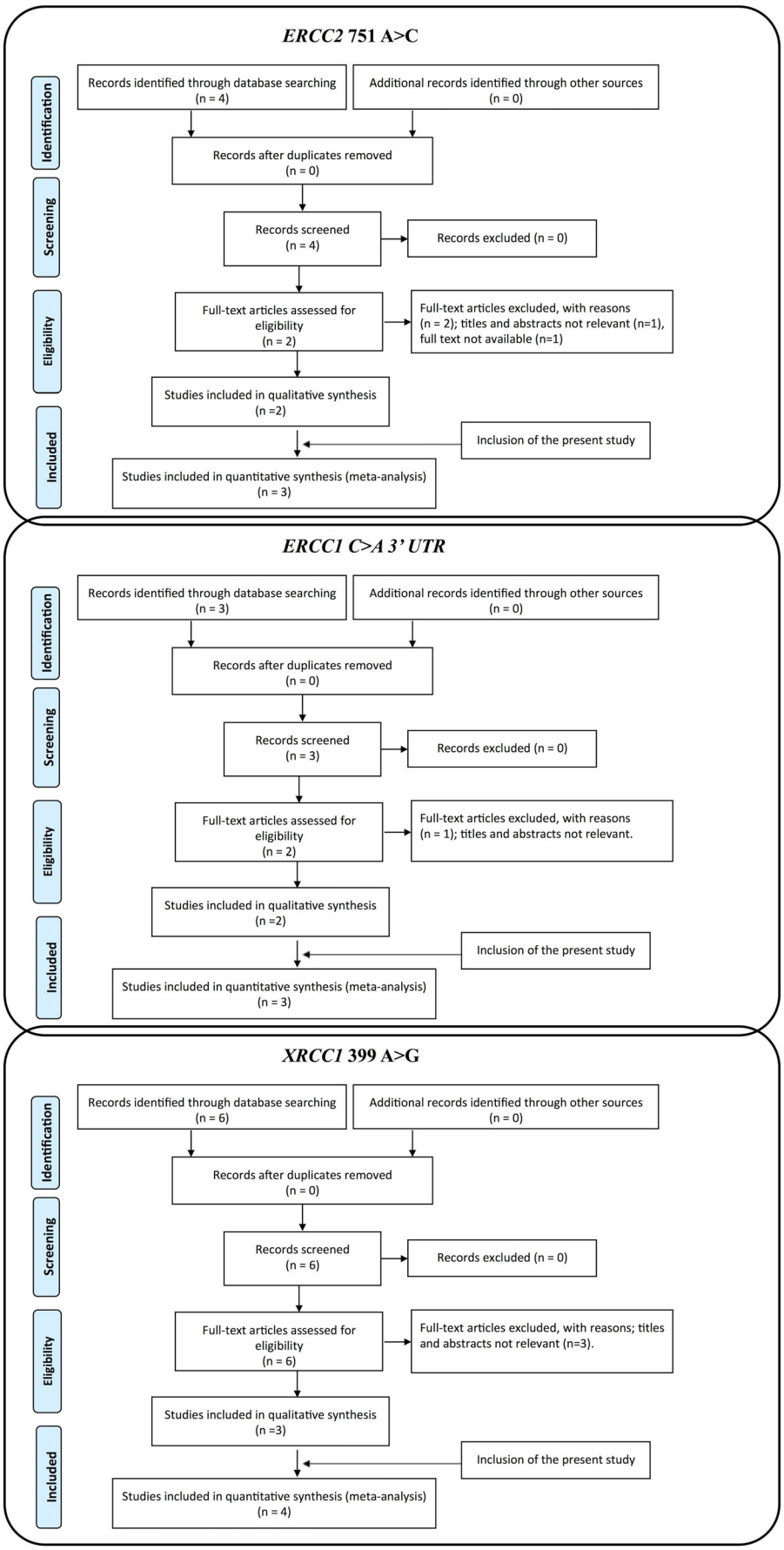


Fig. 1 PRISMA flow diagram. The flow diagrams show screening of literature and selection of studies for meta-analysis

Table 2 Results of meta-analysis using different genetic models

Analysis models	Heterogeneity		Fixed effect		Random effect		Inference
	I^2	P	OR	P	OR	P	
ERCC2 751A > C							
AA vs. (AC + CC)	79.92	0.006	0.948	0.623	1.114	0.706	Not associated
AA vs. AC	71.01	0.031	0.979	0.851	1.090	0.723	Not associated
AA vs. CC	52.44	0.122	0.688	0.702	1.399	0.670	Not associated
AC vs. CC	17.02	0.299	0.794	0.267	0.980	0.963	Not associated
(AA + AC) vs. CC	43.26	0.171	0.732	0.113	1.251	0.746	Not associated
ERCC1 C > A 3′ UTR							
CC vs. (CA + AA)	79.79	0.007	0.994	0.949	1.073	0.757	Not associated
CC vs. CA	64.52	0.059	1.017	0.869	1.071	0.699	Not associated
CC vs. AA	83.13	0.002	0.814	0.270	1.042	0.937	Not associated
CA vs. AA	83.13	0.002	0.814	0.270	1.042	0.937	Not associated
(CC + CA) vs. AA	78.53	0.009	0.825	0.281	0.835	0.453	Not associated
XRCC1 399A > G							
AA vs. (AG + GG)	76.46	0.005	1.001	0.908	1.126	0.553	Not associated
AA vs. AG	75.58	0.006	1.041	0.670	1.170	0.440	Not associated
AA vs. GG	53.73	0.090	0.903	0.568	0.879	0.669	Not associated
AG vs. GG	34.45	0.206	0.887	0.499	0.801	0.367	Not associated
(AA + AG) vs. GG	35.15	0.201	0.892	0.503	0.835	0.453	Not associated

Publication bias

Begg's funnel plot and Egger's regression intercept tests were performed to compute the publication bias. However, no evidence of publication bias was obtained from both tests. The shape of the funnel plot revealed a symmetrical distribution of the studies (Fig. 3), which was confirmed by the Egger's test (Supplementary data 1). The results were further confirmed by classic fail-safe and Orwin's fail-safe test, which suggested no obvious publication bias (Supplementary data 1). With these results, we conclude that the results were stable and the pooled analysis was not biased.

Trial sequential analysis

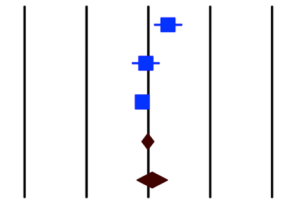
Outcomes of the TSA were concordant with results of meta-analysis and revealed that *XRCC1* 399G > C was not associated with the risk of azoospermia. Moreover, it also revealed that we have yet to reach the adequate number of samples and studies to reach a concrete conclusion as the number of samples did not cross the O'Brien-Fleming boundary (Fig. 4).

Discussion

The impact of polymorphism in the DNA repair pathway genes on male infertility risk has been studied in various populations [4, 7, 21–23]. Most commonly studied SNPs belonged to the *ERCC2*, *ERCC1*, and *XRCC1* genes. SNPs in these genes have indicated an altered DNA repair proficiency in various phenotypic studies [11]. *ERCC2* (751A > C) and *ERCC1* (3′ UTRC > A) SNP have been shown to affect the repair capacity and the tumor's sensitivity to cisplatin [33]. Furthermore, previous researches have reported that SNPs in the *XRCC1* gene may alter the susceptibility towards the risk of thyroid, breast, and bladder cancer [34–36]. We investigated the association between male infertility and four SNPs in *ERCC2* (751A > C), *ERCC1* (3′ UTRC > A) and *XRCC1* (399A > G and 280G > A) genes. We observed that the two alleles at *ERCC2* 751 A > C locus were of comparable frequencies. Genotype comparison using codominant model (AA vs CC) showed that AA genotype increased the risk of infertility in comparison to the CC genotype. This observation is supported by at least two studies, one of which reported that individuals carrying "AA" genotype increased the frequency of X-ray induced chromatid aberrations than those having a "C" allele [37] and the other reported that "C" allele may have higher DNA repair capacity that could effectively decrease the anticancer effect of oxaliplatin [16]. For, *ERCC2* 751A > C, we pooled genotype data on 914 cases and 850 controls from two previously published studies [7, 11] and the present study. The study by Gu et al. reported a significant association of *ERCC2* 751A > C substitution with male infertility. The pooled estimate, however, suggested a lack of association between *ERCC2* 751A > C substitution and male infertility.

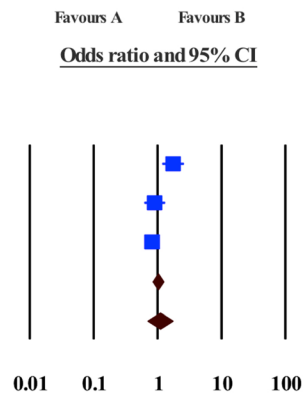
a *ERCC2* 751A>C

Model	Study name	Subgroup within study	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Gu et al. 2007	China	2.086	1.217	3.573	2.676	0.007
	Ji et al. 2008	China	0.911	0.537	1.548	-0.344	0.731
	Present study	India	0.798	0.617	1.033	-1.710	0.087
Fixed			0.948	0.766	1.173	-0.492	0.623
Random			1.114	0.635	1.953	0.377	0.706



b *ERCC1* C>A 3' UTR

Model	Study name	Subgroup within study	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Ji et al. 2008	China	1.750	1.170	2.618	2.725	0.006
	Liu et al. 2017	China	0.901	0.612	1.329	-0.524	0.600
	Present study	India	0.823	0.636	1.065	-1.484	0.138
Fixed			0.994	0.822	1.201	-0.064	0.949
Random			1.073	0.687	1.674	0.309	0.757



c *XRCC1* 399A>G

Model	Study name	Subgroup within study	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value
	Gu et al. 2007	China	0.815	0.549	1.210	-1.012	0.311
	Zheng et al. 2012	North China	2.052	1.227	3.431	2.741	0.006
	Ghasemi et al. 2017	Iran	1.346	0.898	2.018	1.440	0.150
	Present study	India	0.825	0.637	1.067	-1.464	0.143
Fixed			1.011	0.845	1.208	0.116	0.908
Random			1.126	0.761	1.667	0.593	0.553

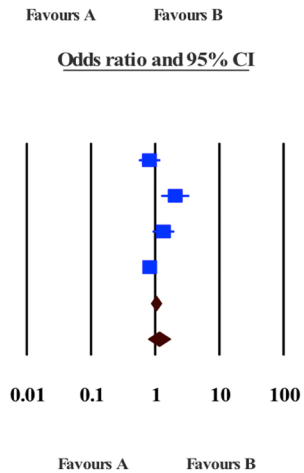
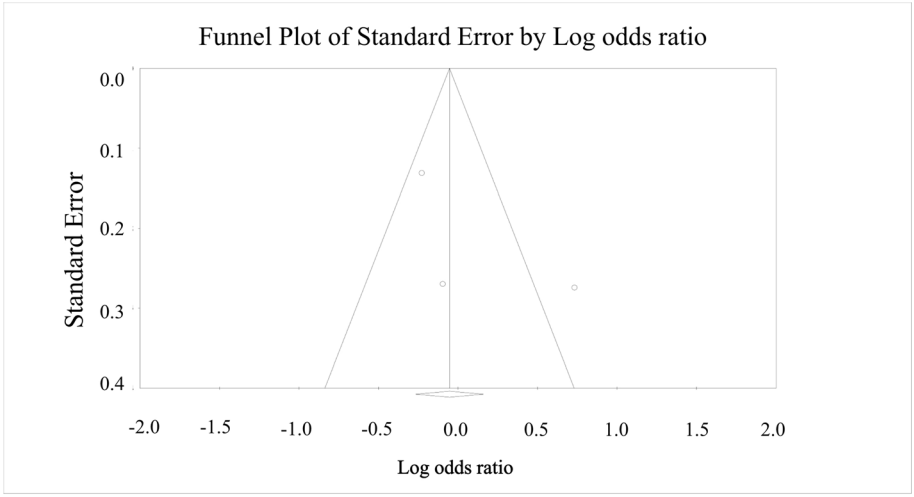


Fig. 2 Forest plot showing the odds ratio, *p* value, and direction of association between *ERCC2* 751A > C, *ERCC1* C > A 3′ UTR, and *XRCC1* 399A > G polymorphism and male infertility

Similarly, for *ERCC1* 3′ UTR C > A, we could retrieve genotype data for 879 cases and 1059 controls from two previously published eligible studies including the present study. While the study by Ji et al. reported a significant association of *ERCC1* 3′ UTR C > A substitution with male infertility [11], Liu et al. reported a complete lack of association [38]. Corresponding to the study by Liu et al., the present case-control study also reflected a lack of association. More studies are however desired to reach any valid conclusion.

For *XRCC1* 399A > G, our case-control study and the meta-analysis suggested a lack of correlation with male infertility risk. Out of the three previously published studies, two reported a lack of association [7, 22]; however, the other study by Zheng et al. reported that A can be

a ***ERCC2* 751A>C**



b ***ERCC1* C>A 3' UTR**

c ***XRCC1* 399A>G**

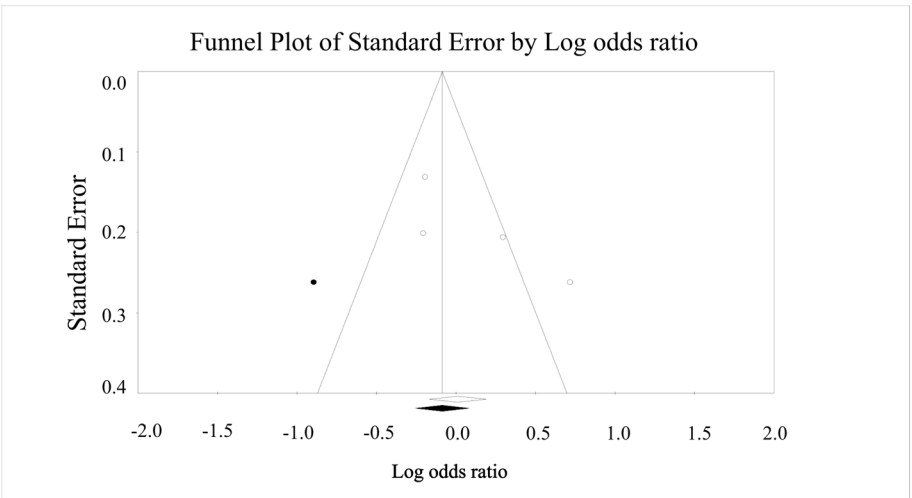


Fig. 3 Funnel plot of precision by log odds ratio showing the absence of publication bias in the dominant model

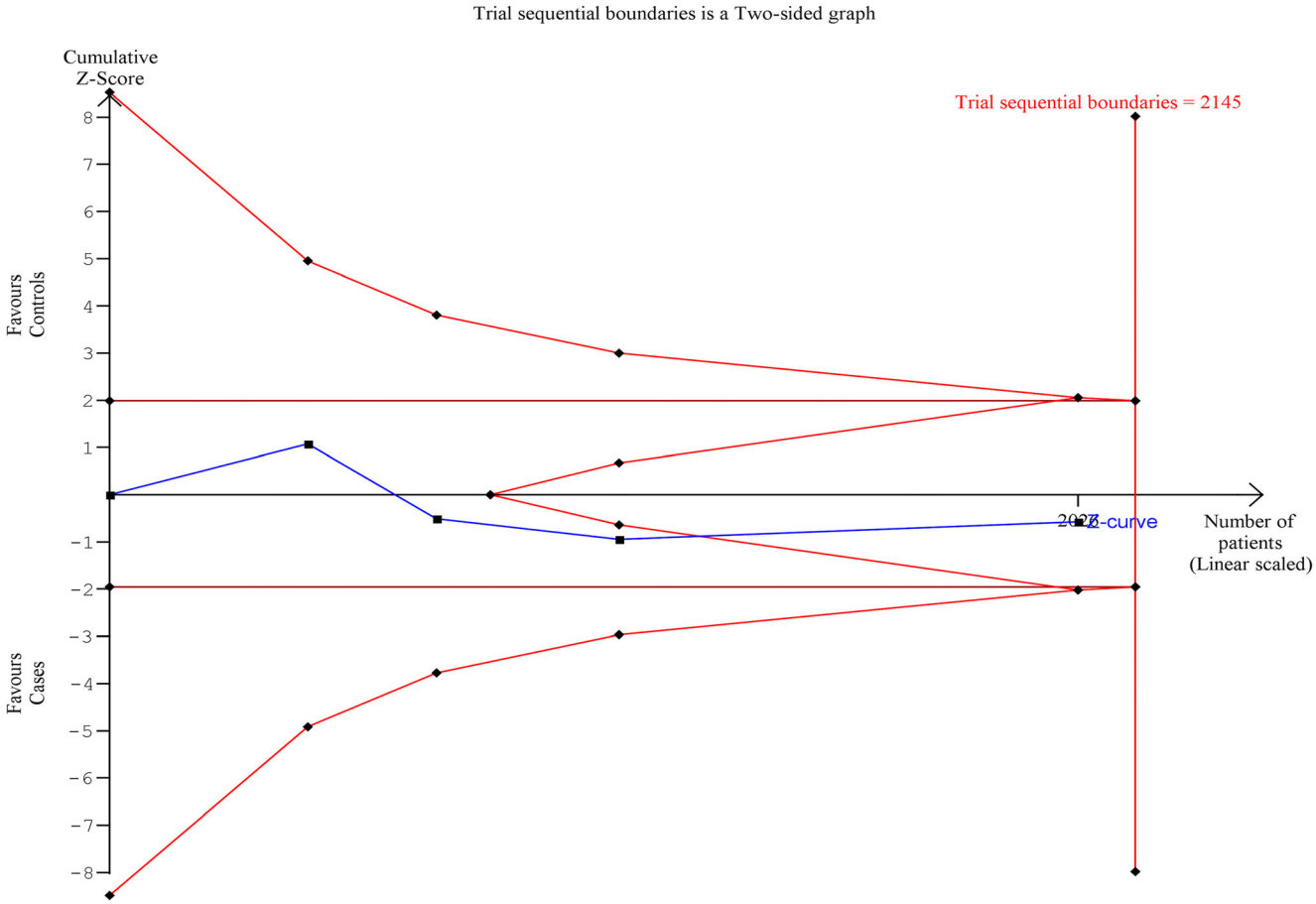


Fig. 4 Trial sequential analysis of the *XRCC1* 399A > G polymorphism and azoospermia risk in the dominant model

considered as a risk allele for idiopathic azoospermia in the northern Han Chinese population [4]. This study, however, carried the smallest sample size among pooled studies. Therefore, sample size may have affected the level of association. We also performed trial sequential analyses to validate the results of meta-analysis. TSA validated this observation for sample size and power and suggested that more studies are required to reach a valid conclusion.

In conclusion, AA genotype at the *ERCC2* 751A > C locus confers a higher risk of infertility in comparison to CC genotype; however, *XRCC1* 399A > G, *XRCC1* 280G > A, and *ERCC1* C > A 3′ UTR substitutions do not correlate with idiopathic azoospermia. Since DNA repair is a complex process involving a heterogeneous complex of proteins, some substitutions may be dependent upon co-existing risk factors. For example, a study evaluated the joint effects of *XRCC1* 399A > G and 280G > A polymorphisms and polycyclic aromatic hydrocarbon exposure on sperm DNA damage and male infertility. They concluded that 399A > G substitution in the *XRCC1* gene alone has a very mild effect on sperm DNA damage and male infertility, while the effect can be increased in the presence of polycyclic aromatic hydrocarbon exposure [21]. Ethnicity-specific associations are another possibility. For example, *XRCC1*, 399A > G, and 194C > T polymorphisms are significant risk factors in the Chinese population [4]. As per our knowledge, this is the first study that assessed the association of *XRCC1* 280G > A, *ERCC1* 3′ UTR C > A, and *ERCC2* 751A > C polymorphisms with azoospermia risk. The strength of the present study is the largest sample size used to analyze the associations of these substitutions with the risk of male infertility. The limitation of the present study includes analysis of selected polymorphisms. Due to significant ethnic variations, the Indian population may have other polymorphisms in the DNA repair genes that correlate with infertility risk. Therefore, further studies are required to establish a correlation between DNA repair gene polymorphisms and infertility.

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Compliance with ethical standards

This study was approved by the Institutional Human Ethics Committee of the Institute of Science, Banaras Hindu University, Varanasi approved this study (Approval letter No. Dean/2011-12/119).

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee.

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

References

1. Huynh T, Mollard R, Trounson A. Selected genetic factors associated with male infertility. Hum Reprod Update Oxford University Press. 2002;8:183–98.
2. Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W. EAU guidelines on male infertility. Eur Urol. 2005; 703–11.
3. Matsuzaka Y, Makino S, Okamoto K, Oka A, Tsujimura A, Matsumiya K, et al. Susceptibility locus for non-obstructive azoospermia is localized within the HLA-DR/DQ subregion: primary role of DQB1* 0604. HLA Wiley Online Library. 2002;60:53–63.
4. Zheng L, Wang X, Zhou D, Zhang J, Huo Y, Tian H. Association between XRCC1 single-nucleotide polymorphisms and infertility with idiopathic azoospermia in northern Chinese Han males. Reprod Biomed Online. Elsevier. 2012;25:402–7.
5. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril Elsevier. 2010;93:1027–36.
6. Gunes S, Al-Sadaan M, Agarwal A. Spermatogenesis, DNA damage and DNA repair mechanisms in male infertility. Reprod Biomed Online. 2015;309–19.
7. Gu A, Ji G, Liang J, Xia Y, Lu N, Wu B, et al. DNA repair gene XRCC1 and XPD polymorphisms and the risk of idiopathic azoospermia in a Chinese population. Int J Mol Med Spandidos Publications. 2007;20:743–7.
8. Jansen J, Olsen AK, Wiger R, Naegeli H, de Boer P, van der Hoeven F, et al. Nucleotide excision repair in rat male germ cells: low level of repair in intact cells contrasts with high dual incision activity in vitro. Nucleic Acids Res Oxford University Press. 2001;29:1791–800.
9. Olsen A-K, Bjørtuft H, Wiger R, Holme J, Seeberg E, Bjørås M, et al. Highly efficient base excision repair (BER) in human and rat male germ cells. Nucleic Acids Res. Oxford University Press. 2001;29:1781–90.
10. Sankhwar M, Sankhwar SN, Bansal SK, Gupta G, Rajender S. Polymorphisms in the XPC gene affect urinary bladder cancer risk: a case-control study, meta-analyses and trial sequential analyses. Sci Rep. Nature Publishing Group. 2016;6:27018.
11. Ji G, Gu A, Xia Y, Lu C, Liang J, Wang S, et al. ERCC1 and ERCC2 polymorphisms and risk of idiopathic azoospermia in a Chinese population. Reprod Biomed Online Elsevier. 2008;17:36–41.
12. Hsia K-T, Millar MR, King S, Selfridge J, Redhead NJ, Melton DW, et al. DNA repair gene Ercc1 is essential for normal spermatogenesis and oogenesis and for functional integrity of germ cell DNA in the mouse. Development The Company of Biologists Ltd. 2003;130:369–78.
13. Thompson LH, Brookman KW, Jones NJ, Allen SA, Carrano AV. Molecular cloning of the human XRCC1 gene, which corrects defective DNA strand break repair and sister chromatid exchange. Mol Cell Biol. 1990;10:6160–71 Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=362891&tool=pmcentrez&rendertype=abstract.
14. Ahmed EA, de Boer P, Philippens MEP, Kal HB, de Rooij DG. Parp1–XRCC1 and the repair of DNA double strand breaks in mouse round spermatids. Mutat Res Mol Mech Mutagen Elsevier. 2010;683:84–90.
15. Geredeli C, Artac M, Yildirim S, Inal A, Dede I, Guler T, et al. Prognostic value of ERCC1, ERCC2, XRCC1, and TP53 single nucleotide polymorphisms in patients with early-stage non-small cell lung cancer. Tumor Biol Springer. 2015;36:4279–85.
16. Yin M, Yan J, Martinez-Balibrea E, Graziano F, Lenz H-J, Kim H-J, et al. ERCC1 and ERCC2 polymorphisms predict clinical outcomes of oxaliplatin-based chemotherapies in gastric and colorectal cancer: a systemic review and meta-analysis. Clin Cancer Res. AACR. 2011;17:1632–40.
17. Yang B, Wang H, Gao X-K, Chen B-Q, Zhang Y-Q, Liu H-L, et al. Expression and significance of Rap1A in testes of azoospermic subjects. Asian J Androl. 2004;6:35–40.
18. Okuda K, Sasaki H, Hikosaka Y, Kawano O, Yukiue H, Yano M, et al. Excision repair cross complementation group 1 polymorphisms predict overall survival after platinum-based chemotherapy for completely resected non-small-cell lung cancer. J Surg Res Elsevier. 2011;168:206–12.
19. Matullo G, Peluso M, Polidoro S, Guarrera S, Munnia A, Krogh V, et al. Combination of DNA repair gene single nucleotide polymorphisms and increased levels of DNA adducts in a population-based study. Cancer Epidemiol Prev Biomarkers. AACR. 2003;12:674–7.
20. Dhillon VS, Yeoh E, Fenech M. DNA repair gene polymorphisms and prostate cancer risk in South Australia—results of a pilot study. Urol Oncol Semin Orig Investig, Elsevier. 2011:641–6.
21. Ji G, Gu A, Zhu P, Xia Y, Zhou Y, Hu F, et al. Joint effects of XRCC1 polymorphisms and polycyclic aromatic hydrocarbons exposure on sperm DNA damage and male infertility. Toxicol Sci Oxford University Press. 2010;116:92–8.
22. Ghasemi H, Khodadadi I, Fattahi A, Moghimbeigi A, Tavilani H. Polymorphisms of DNA repair genes XRCC1 and LIG4 and idiopathic male infertility. Syst Biol Reprod Med. Taylor & Francis. 2017;63:382–90.
23. Liu CL, Jiao HY, Ma Q, Wu X, Jia ST, Jing WH, et al. Relationship between ERCC2 single nucleotide polymorphisms and male idiopathic infertility in Ningxia. Zhonghua nan ke xue= Natl J Androl. 2014;20:419–22.
24. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16:231–45 Available from: http://www.ncbi.nlm.nih.gov/pubmed/19934213.
25. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl cancer Inst. Oxford University Press. 1959;22:719–48.
26. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials Elsevier. 1986;7:177–88.
27. Brok J, Thorlund K, Wetterslev J, Gluud C. Apparently conclusive meta-analyses may be inconclusive—trial sequential analysis adjustment of random error risk due to repetitive testing of accumulating data in apparently conclusive neonatal meta-analyses. Int J Epidemiol Oxford University Press. 2008;38:287–98.
28. Higgins JPT, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. British Medical Journal Publishing Group. 2011;343:d5928.
29. Bansal SK, Jaiswal D, Gupta N, Singh K, Dada R, Sankhwar SN, et al. Gr/gr deletions on Y-chromosome correlate with male

infertility: an original study, meta-analyses, and trial sequential analyses. Sci Rep. Nature Publishing Group. 2016;6:19798.
30. Carlus SJ, Sarkar S, Bansal SK, Singh V, Singh K, Jha RK, et al. Is MTHFR 677 C> T polymorphism clinically important in polycystic ovarian syndrome (PCOS)? A case-control study, meta-analysis and trial sequential analysis. PLoS One. Public Library of Science. 2016;11:e0151510.
31. Tsuchiya N, Mishina M, Narita S, Kumazawa T, Inoue T, Horikawa Y, et al. Association of XRCC1 gene polymorphisms with the susceptibility and chromosomal aberration of testicular germ cell tumors. Int J Oncol Spandidos Publications. 2006;28:1217–23.
32. Mirsane SA, Oraei N. Alcohol consumption and polymorphism of XRCC1 gene: two serious dangers for males' fertility. Nephrourol Mon Kowsar. 2017;9.
33. Rosell R, Taron M, Ariza A, Barnadas A, Mate JL, Reguart N, et al. Molecular predictors of response to chemotherapy in lung cancer. Semin Oncol. Elsevier. 2004:20–7.
34. Moullan N, Cox DG, Angèle S, Romestaing P, Gérard J-P, Hall J. Polymorphisms in the DNA repair gene XRCC1, breast cancer risk, and response to radiotherapy. Cancer Epidemiol Prev Biomarkers AACR. 2003;12:1168–74.
35. Sak SC, Barrett JH, Paul AB, Bishop DT, Kiltie AE. DNA repair gene XRCC1 polymorphisms and bladder cancer risk. BMC Genet. BioMed Central. 2007;8:13.
36. Yan L, Li Q, Li X, Ji H, Zhang L. Association studies between XRCC1, XRCC2, XRCC3 polymorphisms and differentiated thyroid carcinoma. Cell Physiol Biochem Karger Publishers. 2016;38:1075–84.
37. Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, et al. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis Oxford University Press. 2000;21:551–5.
38. Liu S-Y, Zhang C-J, Peng H-Y, Sun H, Lin K-Q, Huang X-Q, et al. Strong association of SLC1A1 and DPF3 gene variants with idiopathic male infertility in Han Chinese. Asian J Androl. Medknow Publications. 2017;19:486.