Journal of Population Therapeutics
& Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jms3zk05

Impact of DNA repair gene variants on male infertility susceptibility in south Indian population

M. Venkateswara Rao[1]*, K. V. N. Geetha Devi[2], D. Rajarajeswari[3], K. Ashalatha[4]

[1] *Department of Anatomy, Narayana Medical College, Nellore, Andhra Pradesh, India.
[2]Department of Anatomy, ACSR Government Medical College, Dargamitta, Nellore,
Andhra Pradesh, India.
[3] Department of Biochemistry, Narayana Medical College, Nellore, Andhra Pradesh, India.
[4] Department of Anatomy, Narayana Medical College, Nellore, Andhra Pradesh, India.

***Corresponding author:** Dr. M. Venkateswara Rao
*Department of Anatomy, Narayana Medical College, Nellore, Andhra Pradesh, India.

ABSTRACT

Aim: This study investigated the association of polymorphisms in four key DNA repair genes—XRCC1 (rs25487), XRCC3 (rs861539), XPD (rs13181), and APEX1 (rs1130409)—with male infertility risk in a South Indian population cohort.

Materials and Methods: A total of 150 male participants (100 infertile patients and 50 fertile controls) were included in a case–control study design. Genotyping of target loci was performed using the PCR-RFLP method, and genotype–phenotype associations were analyzed under codominant, dominant, and recessive inheritance models.

Results: A significant association was observed between the XRCC1 rs25487 AG genotype and male infertility risk (OR = 3.22, p = 0.02), indicating a potential heterozygote effect. XPD rs13181 AC genotype exhibited a strong correlation with infertility (OR = 5.81, $p < 0.0001$), suggesting that reduced nucleotide excision repair efficiency may increase susceptibility. In contrast, XRCC3 rs861539 showed no significant association under any genetic model. Notably, all infertile subjects carried the APEX1 rs1130409 GG genotype, whereas the TT genotype was exclusively detected among fertile controls, indicating a strong genetic predisposition likely linked to impaired base excision repair function.

Conclusion: The findings highlight the pivotal role of DNA repair gene polymorphisms—particularly in XRCC1, XPD, and APEX1—in modulating male infertility risk. These results underscore the potential value of incorporating genetic screening into infertility diagnostics and warrant further large-scale, multi-ethnic, and functional studies to elucidate the underlying molecular mechanisms.

Keywords: DNA repair genes; Genetic polymorphism; Male infertility

INTRODUCTION

Infertility is a significant global public health concern, affecting approximately 15% of couples worldwide, with male factors contributing to nearly half of all cases (**Agarwal et al., 2015; Mascarenhas et al., 2012**). Male infertility is a multifactorial condition arising from a range of causes, including genetic abnormalities, environmental exposures, hormonal imbalances, lifestyle influences, and anatomical defects (**Krausz & Riera-Escamilla, 2018**). Clinically, it manifests through various semen abnormalities such as oligozoospermia, azoospermia, asthenozoospermia, or teratozoospermia. Despite the widespread use of assisted reproductive technologies (ART), including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), the underlying etiology remains unexplained in up to 40% of infertile men (**Krausz, 2011; Barratt et al., 2017**). This high rate of idiopathic infertility underscores the need for a deeper understanding of the molecular and genetic mechanisms involved, particularly those related to genome stability and DNA repair.

Genomic integrity plays a vital role in spermatogenesis, with processes such as chromatin remodeling, meiotic recombination, and DNA repair being critical for the production of functionally competent sperm. Disruptions in these pathways can lead to increased sperm DNA fragmentation (SDF), chromosomal abnormalities, and impaired fertilization potential (**Zini & Libman, 2006; Aitken & De Iuliis, 2010**). Elevated SDF is now recognized as a biomarker for male infertility, associated with reduced sperm quality, impaired embryo development, and higher miscarriage rates (**Simon et al., 2017; Esteves et al., 2021**). Although oxidative stress is a known contributor to SDF, the intrinsic DNA repair capacity of germ cells also plays a key role in maintaining sperm DNA integrity.

Multiple DNA repair mechanisms are active during spermatogenesis, notably base excision repair (BER), nucleotide excision repair (NER), and homologous recombination repair (HRR). Polymorphisms in genes involved in these pathways may compromise repair efficiency, resulting in the accumulation of DNA damage and contributing to infertility (**Singh & Jaiswal, 2022; Lu et al., 2020**). Among these, polymorphisms in XRCC1 (rs25487), XRCC3 (rs861539), XPD/ERCC2 (rs13181), and APEX1 (rs1130409) have been widely studied. These variants have been implicated in defective DNA repair, increased SDF, and abnormal sperm parameters, though results vary across populations and study designs.

Despite mounting evidence, key knowledge gaps persist. Most studies have focused on single gene associations without integrating genotypic data with detailed phenotypic profiles or infertility subtypes.

The present study aims to investigate the association between polymorphisms in XRCC1, XRCC3, XPD, and APEX1 and male infertility in a South Indian cohort.

Materials and methods

The prospective case–control study was conducted in the Department of Anatomy, Narayana Medical College, Nellore, Andhra Pradesh, India. Written informed consent was obtained from all participants in accordance with institutional and national ethical guidelines. The study protocol was approved by the Institutional Ethics Committee of Narayana Medical College, Nellore.

Study population and sample size

A total of 150 male participants were enrolled in the study, including 100 infertile men (cases) and 50 age-matched fertile men (controls).

Inclusion criteria

- Men aged 25–60 years.
- Infertile men with ≥2 years of unprotected intercourse without conception.
- Physically and mentally healthy males.
- Fertile controls with no history of infertility, co-morbidities, prostate cancer, or HIV infection.

Exclusion criteria

- Men with prostate cancer, erectile dysfunction, venereal diseases, varicocele, or significant co-morbidities (e.g., diabetes mellitus, renal disease, hypothyroidism).
- Patients with congenital anomalies of genital organs (hypospadias), Klinefelter's syndrome, hypogonadotropic hypogonadism, Y-chromosome microdeletions, or obstructive azoospermia.
- History of cryptorchidism, vasectomy, mumps orchitis, cytotoxic drug use, or irradiation.

- History of malignancy or treatment with chemotherapy.

Genotyping of DNA repair genes

Genomic DNA was extracted using the phenol–chloroform method.[19]

Genotyping of four DNA repair gene polymorphisms was performed using PCR–RFLP:

- XRCC1 (Arg399Gln, rs25487, exon 10)
- XRCC3 (Thr241Met, rs861539, exon 7)
- XPD (Lys751Gln, rs13181, exon 23)
- APEX1 (Asp148Glu, rs1130409, exon 5)

PCR amplification of the target regions was carried out in a 25 µL reaction volume containing ~100 ng of genomic DNA, 0.2 µM of each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1× PCR buffer, and 1 U of Taq DNA polymerase.

Table 1: Oligonucleotide sequences used for genotyping different DNA repair gene polymorphisms

	Primer sequences
XRCC1 (rs25487) genotyping	
Forward primer (5' to 3')	ACCTTGTGCTTTCTCTGTGTC
Reverse primer (5' to 3')	TAGTCTGCTGG CTCTGGGCT
XRCC3 (rs861539) genotyping	
Forward primer (5' to 3')	GACACCTTGTTGGAGTGTGT
Reverse primer (5' to 3')	GTCTTCTCGATGGTTAGGCA
XPD (rs13181) genotyping	
Forward primer (5' to 3')	GCCCGCTCTGGATTATACG
Reverse primer (5' to 3')	CTATCATCTCCTGGCCCCC
APEX1 (rs1130409) genotyping	
Forward primer (5' to 3')	CCAGCTGAACTTCAGGAGCT
Reverse primer (5' to 3')	CTCGGCCTGCATTAGGTACA

Table 2: Details of PCR conditions for amplifying different gene polymorphic DNA sequences

	Initial denaturation	**Denaturation**	**Annealing**	**Extension**	**Final extension**
XRCC1 (rs25487)	95 °C for 3 min	94 °C for 30 sec	57 °C for 45 sec	72 °C for 45 sec	72 °C for 5 min
No. of cycles	1	40			1
XRCC3 (rs861539)	95 °C for 5 min	94 °C for 30 sec	55 °C for 30 sec	72 °C for 30 sec	72 °C for 10 min
No. of cycles	1	40			1
XPD (rs13181)	94 °C for 3 min	94 °C for 45 sec	60 °C for 45 sec	72 °C for 60 sec	72 °C for 7 min
No. of cycles	1	38			1
APEX1 (rs1130409)	94 °C for 5 min	94 °C for 30 sec	55 °C for 30 sec	72 °C for 30 sec	72 °C for 10 min
No. of cycles	1	35			1

XRCC1 (rs25487): NciI RFLP analysis

PCR products were digested with the NciI (recognition sequence: CC/GG) by incubating 10 µL of amplified product with 5 U of the enzyme in a 20 µL reaction mixture at 37 °C for 4 h, following the manufacturer's instructions. Digested fragments were resolved on agarose gel alongside a 100 bp DNA ladder. The Arg allele at codon 399 introduces an NciI recognition site, producing fragments of 387 bp and 137 bp, whereas the Gln allele lacks this site and remains uncut at 524 bp. Genotypes were identified as Arg/Arg (two bands), Arg/Gln (three bands), or Gln/Gln (single band).

XRCC3 (rs861539): FatI RFLP analysis

PCR products were digested with FatI (recognition sequence: CC/TT) by incubating 10 µL of amplified product with 5 U of enzyme in a 20 µL reaction mixture overnight, following the manufacturer's instructions. Digested fragments were resolved on agarose gel alongside a 100 bp DNA ladder. The T allele at codon 358 creates a FatI recognition site, producing fragments of 200 bp and 158 bp, whereas the C allele remains uncut at 358 bp. Genotypes were identified as CC (single band), CT (three bands), or TT (two bands).

XPD (rs13181): PstI RFLP analysis

PCR products were digested with the PstI (recognition sequence: AA/CC) by incubating 10 µL of amplified product with 5 U of enzyme in a 20 µL reaction mixture overnight, following the manufacturer's instructions. Digested fragments were resolved on a 4% agarose gel and visualized alongside a 100 bp DNA ladder. Genotypes were assigned based on fragment patterns: AA genotype produced two fragments (290 bp and 146 bp), AC genotype produced four fragments (290, 227, 146, and 63 bp), and CC genotype generated three fragments (227, 146, and 63 bp).

APEX1 (rs1130409): MnlI RFLP analysis

PCR products were digested with the MnlI (recognition sequence: TT/GG) by incubating 10 µL of amplified product with 2 U of enzyme in a 20 µL reaction mixture at 37 °C for 2 h, according to the manufacturer's protocol. Digested fragments were resolved on a 3% agarose gel alongside a 100 bp DNA ladder. The T allele lacks the MnlI recognition site, remaining undigested at 350 bp fragment, whereas the G allele introduces the site, producing two fragments of 252 bp and 98 bp. Genotypes were assigned as TT (350 bp), TG (350, 252, and 98 bp), and GG (252 and 98 bp).

Genotype data were also analyzed under three genetic models:

- **Codominant model:** Each genotype (e.g., AA, AG, and GG) analyzed separately.
- **Dominant model:** Heterozygotes and minor allele homozygotes grouped and compared to major allele homozygotes.
- **Recessive model:** Minor allele homozygotes compared to combined group of heterozygotes and major allele homozygotes.

Statistical analysis

All statistical analyses were performed using SPSS software (version 23.0). Statistical significance was defined as a two-tailed p value < 0.05, and multiple testing corrections were applied where appropriate. Genotype and allele frequencies were calculated for both cases and controls, and conformity with Hardy–Weinberg equilibrium was assessed. Multiple genetic models, including codominant, dominant, and recessive models, were applied to comprehensively assess the effects of each variant under different inheritance assumptions.

Results

XRCC1 (rs25487) genotype and allele frequency distribution

The genotype frequencies of AA, AG, and GG were compared between cases and controls. The heterozygous genotype (AG) was more prevalent in cases (59%) compared to controls (28%), while the homozygous GG genotype was more frequent in controls (46%) than in cases (24%). The AA genotype was observed in 17% of cases and 26% of controls (Table 3). Statistical analysis revealed a significant association between the AG genotype and disease risk. Individuals with the AG genotype had an odds ratio (OR) of 3.22 (95% CI: 1.27–8.15; *p* = 0.02), indicating more than a threefold increased likelihood of disease compared to individuals with the AA genotype. In contrast, the GG genotype did not show a significant association with disease susceptibility (OR = 0.79, 95% CI: 0.32–2.00; *p* = 0.80).

Table 3: Genotype distribution and association analysis between cases and controls

Genotypes	Cases	Controls	OR	95% CI	*p* value

	(N=100)	(N=50)			
AA	17	13			
AG	59	14	3.22	1.27 - 8.15	0.02
GG	24	23	0.79	0.32 - 2.00	0.8

Genetic model consideration

Under the **dominant model** (AG+GG vs. AA), carriers of at least one G allele showed a significantly increased disease risk (OR = 2.43, 95% CI = 1.04–5.68, *p* = 0.04) (Table 4). In contrast, the recessive model (GG vs. AG+AA) revealed no significant association (OR = 0.66, 95% CI = 0.31–1.41, *p* = 0.28), indicating that homozygosity for the G allele does not elevate risk. The co-dominant model demonstrated a heterozygote effect: the AG genotype was significantly associated with increased risk (OR = 3.22, 95% CI = 1.27–8.15, *p* = 0.02), while the GG genotype showed no difference compared with AA (OR = 0.79, 95% CI = 0.32–2.00, *p* = 0.80). Collectively, these results suggest that disease susceptibility is primarily driven by the heterozygous AG genotype rather than GG homozygosity.

Table 4: Association of genotypes with disease risk under different genetic models

Genetic Model	Genotype Comparison	Cases (N=100)	Controls (N=50)	OR	95% CI	*p* value
Dominant	AG + GG vs. AA	83	37	2.43	1.04 – 5.68	0.04
Recessive	GG vs. AG + AA	24	23	0.66	0.31 – 1.41	0.28
Co-dominant	AG vs. AA	59 vs. 17	14 vs. 13	3.22	1.27 – 8.15	0.02
	GG vs. AA	24 vs. 17	23 vs. 13	0.79	0.32 – 2.00	0.80

Hardy-Weinberg Equilibrium and allele frequency analysis

To evaluate whether genotype distributions in cases and controls deviate from expected genetic equilibrium, a Hardy-Weinberg equilibrium (HWE) analysis was performed for both groups, along with estimation of allele frequencies (Table 5). HWE analysis revealed a significant deviation among controls (*p* = 0.003), with observed genotype frequencies differing from those expected under equilibrium conditions. In contrast, cases showed no significant deviation (*p* = 0.06). Allele frequencies were 0.40 (A) and 0.60 (G) in controls, and 0.46 (A) and 0.54 (G) in cases. These findings indicate that the control group deviated from HWE, whereas the case group remained in approximate equilibrium.

Table 5: HWE analysis of cases and controls

HWE				**Allele frequency**		
	AA	AG	GG	A	G	P
Controls (N=50)						
Observed	13	14	23	0.4	0.6	0.003
Expected	8	24	18			
Cases (N=100)						
Observed	17	59	24	0.46	0.54	0.06
Expected	21.6	49.8	28.6			

XRCC3 (rs861539) genotype and allele frequency distribution

Genotype association analysis showed no significant differences between cases and controls (Table 6). The CC genotype was the most frequent in both groups (73% in cases vs. 68% in controls) and served as the reference. The CT genotype occurred in 24% of cases and 24% of controls, with no significant association (OR = 0.93, 95% CI = 0.42–2.08, *p* = 1.0). The TT genotype was rare, observed in 3% of cases and 8% of controls, and also showed no significant association (OR = 0.35, 95% CI = 0.07–1.64, *p* = 0.33). These results indicate that neither heterozygous nor homozygous T alleles confer increased disease risk compared to the CC genotype.

Table 6: Distribution of CC, CT, and TT genotypes in cases and controls with corresponding odds ratios (OR), 95% confidence intervals (CI), and p values. The CC genotype was used as the reference.

Genotypes	Cases (N=100)	Controls (N=50)	OR	CI	*p* value
CC	73	34			
CT	24	12	0.93	0.42 - 2.08	1
TT	3	4	0.35	0.07 -1.64	0.33

Genetic model consideration

Analysis under different inheritance models showed no significant associations with disease risk (Table 7). In the dominant model (CT+TT vs. CC), carriers of the T allele did not show increased risk (OR = 0.79, 95% CI = 0.38–1.66, *p* = 0.52). Similarly, the recessive model (TT vs. CC+CT) indicated no significant effect of TT homozygosity (OR = 0.36, 95% CI = 0.08–1.66, *p* = 0.17). In the co-dominant model, neither the CT genotype (OR = 0.93, 95% CI = 0.42–2.08, *p* = 0.87) nor the TT genotype (OR = 0.35, 95% CI = 0.07–1.65, *p* = 0.17) differed significantly from CC. Collectively, these findings suggest that the T allele does not contribute to disease susceptibility under any of the tested models.

Table 7: Association of genotypes with disease risk under dominant, recessive, and co-dominant models

Genetic Model	Genotype Groups	OR	95% CI	Chi-square	*p* value
Dominant	CT + TT vs CC	0.79	0.38 – 1.66	0.41	0.52
Recessive	TT vs CC + CT	0.36	0.08 – 1.66	1.89	0.17
Co-dominant	CC vs CT	0.93	0.42 – 2.08	0.03	0.87
	CC vs TT	0.35	0.07 – 1.65	1.92	0.17

Hardy-Weinberg Equilibrium and allele frequency analysis

HWE analysis showed no significant deviation in either group (Table 8). Among controls, the observed genotype distribution (CC = 34, CT = 12, TT = 4) was consistent with expectations (p = 0.07), with allele frequencies of 0.80 (C) and 0.20 (T). Similarly, cases (CC = 73, CT = 24, TT = 3) did not deviate from HWE (*p* = 0.56), with allele frequencies of 0.85 (C) and 0.15 (T). These results indicate that both case and control populations were in equilibrium, supporting the reliability of the genotyping data.

Table 8: HWE analysis of cases and controls with observed and expected genotype frequencies, allele frequencies, and p values.

HWE				**Allele frequency**		
	CC	CT	TT	C	T	*p* value
Controls (N=50)						
Observed	34	**12**	4	0.8	0.2	0.07
Expected	32	16	2			
Cases (N=100)						
Observed	73	24	3	0.85	0.15	0.56
Expected	72.3	25.5	2.3			

XPD (rs13181) genotypic variants

Genotype association analysis demonstrated a significant difference between cases and controls (Table 9). The AC genotype was markedly more frequent in cases (50%) than in controls (16%), conferring a significantly increased risk of disease (OR = 5.81, 95% CI = 2.45–13.76, p = 0.00004). The CC genotype was rare in both groups (7% in cases, 4% in controls) and showed no significant association (OR = 3.25, 95% CI = 0.63–16.60, p = 0.25). The AA genotype was used as the reference. These findings suggest that heterozygosity for the AC genotype is strongly associated with disease susceptibility, whereas CC homozygosity does not significantly influence risk.

Table 9: Genotype distribution in cases and controls with corresponding odds ratios (OR), 95% confidence intervals (CI), and p values

Genotypes	Cases (N=100)	Controls (N=50)	OR	95% CI	*p* value
AA	43	40			
AC	50	8	5.81	2.45 - 13.76	0.00004
CC	7	2	3.25	0.63 - 16.60	0.25

Genetic model consideration

Analysis across different genetic models revealed distinct trends (Table 10). The dominant model (AC+CC vs AA) showed a significant association with disease risk, with an OR of 5.30 (95% CI: 2.50–11.22; p < 0.0001), indicating that carriers of at least one C allele had markedly higher susceptibility compared to AA homozygotes. In the recessive model (CC vs AA+AC), the association was not significant (OR = 1.81; 95% CI: 0.33–9.90; p = 0.50). In the co-dominant model, AC vs AA showed a strong and significant effect (OR = 5.81; 95% CI: 2.45–13.76; p = 0.00004), whereas CC vs AA comparison did not reach statistical significance (OR = 3.25; 95% CI: 0.63–16.60; p = 0.25). Overall, the dominant and heterozygous co-dominant models indicated a robust association between the polymorphism and increased disease risk, while the recessive and homozygous co-dominant models did not.

Table 10: Association of genotypes with disease risk under dominant, recessive, and co-dominant models

Genetic Model	Cases	Controls	OR	95% CI	*p* value
Dominant (AC+CC vs AA)	57	10	5.30	(2.50 – 11.22)	<0.0001
Recessive (CC vs AA+AC)	7	2	1.81	(0.33 – 9.90)	0.50
Co-dominant (AC vs AA)	50	8	5.81	2.45 – 13.76	0.00004
	7	2	3.25	0.63 – 16.60	0.25

Hardy-Weinberg Equilibrium and allele frequency analysis

HWE analysis demonstrated that both cases and controls were in approximate equilibrium (Table 11). Among controls, the observed genotype distribution (AA = 40, AC = 8, CC = 2) did not differ significantly from the expected frequencies (p = 0.08), with allele frequencies of 0.88 (C) and 0.12 (T). Similarly, in cases, the observed distribution (AA = 43, AC = 50, CC = 7) was consistent with expectations (p = 0.13), with allele frequencies of 0.68 (C) and 0.32 (T). These results indicate no significant deviation from HWE in either group, supporting the reliability of the genotyping data.

Table 11: HWE analysis of cases and controls with observed and expected genotype frequencies, allele frequencies, and p values

HWE				**Allele frequency**		
	AA	**AC**	**CC**	C	T	P
Controls (N=50)						
Observed	40	8	2	0.88	0.12	0.08

Expected	38.7	10.6	0.7			
Cases (N=100)						
Observed	43	50	7	0.68	0.32	0.13
Expected	46.2	43.5	10.2			

APEX1 (rs1130409) genotypic variants

Genotype distribution analysis showed a striking difference between cases and controls . All cases (100%) carried the GG genotype, whereas none exhibited GT or TT genotypes. In contrast, controls displayed greater variability, with 23% having the GG genotype and 22% the TT genotype, while no GT heterozygotes were detected. This pattern suggests a strong association of the GG genotype with disease susceptibility.

Genetic model consideration

Genotype association analysis revealed a striking difference between cases and controls (Table 12). All cases carried the GG genotype (100%), whereas controls displayed a mix of GG (46%) and TT (44%) genotypes, with no heterozygotes observed. Under dominant, recessive, and co-dominant models, the odds ratios were effectively infinite, with Fisher's exact tests indicating highly significant associations (p < 0.0001).

Table 12: Genotype association under dominant, recessive, and co-dominant models. Odds ratios were infinite, and Fisher's exact test showed significant.

Genetic Model	Cases (N=100)	Controls (N=50)	OR	95% CI	*p* value
Dominant (GG+GT vs TT)	100 vs 0	23 vs 22	∞	NA (Fisher's exact)	<0.0001
Recessive (GG vs GT+TT)	100 vs 0	23 vs 27	∞	NA (Fisher's exact)	<0.0001
Co-dominant (GG vs TT)	100 vs 0	23 vs 22	∞	NA (Fisher's exact)	<0.0001

HWE analysis

HWE analysis showed that the case group was monomorphic, rendering HWE testing inapplicable, while the control group deviated significantly from equilibrium due to the absence of GT heterozygotes (p < 0.0001, Table 13).

Table 13: HWE analysis table showing observed and expected genotypes, allele frequencies, and *p* values for both cases and controls

Group	Observed (AA/AC/CC)	Expected (AA/AC/CC)	Allele Frequency (A/C)	*p* value
Controls (N=50)	40 / 8 / 2	38.7 / 10.6 / 0.7	0.88 / 0.12	0.08
Cases (N=100)	43 / 50 / 7	46.2 / 43.5 / 10.2	0.68 / 0.32	0.13

DISCUSSION

In this study, we investigated the role of polymorphisms in four key DNA repair genes (XRCC1 rs25487, XRCC3 rs861539, XPD rs13181, and APEX1 rs1130409) and their association with disease risk. Our findings demonstrate distinct genotype-specific patterns, highlighting both risk-enhancing and potentially protective effects.

For XRCC1 (rs25487), the AG genotype was significantly associated with disease susceptibility, conferring more than a threefold increased risk compared to AA carriers. The absence of association with the GG genotype suggests a possible overdominant effect, in which heterozygotes are at higher risk while homozygotes for the variant allele are not. This finding is consistent with prior research implicating XRCC1 polymorphisms in impaired base excision repair efficiency, particularly in heterozygous states that may lead to imbalanced protein interactions (**Berwick & Vineis, 2000; Hoeijmakers, 2001**).

In contrast, XRCC3 (rs861539) did not show a significant association under any genetic model. The predominance of the CC genotype across cases and controls, along with the absence of significant effects for CT or TT genotypes, suggests that this variant may not be a major contributor to disease risk in this cohort. Although the TT genotype indicated a trend toward reduced risk, the wide confidence intervals highlight the need for larger studies to clarify its potential protective role (**Shen et al., 1998; Romanowicz-Makowska et al., 2012**).

XPD (rs13181) emerged as another strong candidate, with carriers of the AC genotype exhibiting a markedly increased risk of disease (OR = 5.81). The dominant model (AC+CC vs AA) confirmed a significant association, underscoring the role of the C allele in modulating susceptibility. Previous studies have reported that XPD variants alter nucleotide excision repair capacity, leading to increased genomic instability and heightened cancer susceptibility **(Spitz et al., 2001**). While the CC genotype also suggested increased risk, the small number of carriers limited statistical power, reinforcing the need for validation in larger cohorts.

Finally, APEX1 (rs1130409) revealed striking differences: all cases carried the GG genotype, whereas TT was observed exclusively in controls. This clear divergence strongly suggests the GG genotype as a risk factor, while the TT genotype may exert a protective effect. The absence of heterozygotes in both groups could reflect population-specific genetic architecture. Prior studies have highlighted the central role of APEX1 in base excision repair, where functional variants can disrupt repair efficiency and influence disease susceptibility (**Hu et al., 2001; Abhishek et al., 2019**).

Several limitations warrant consideration. The sample size was modest, which reduced statistical power for less frequent genotypes (e.g., XPD-CC, XRCC3-TT) and may have contributed to wide confidence intervals. The absence of the APEX1 GT genotype limited assessment of allelic interactions and suggests possible population-specific allele distributions.

CONCLUSION

In conclusion, this study demonstrated that genetic variability in DNA repair pathways significantly influences disease susceptibility. Specifically, XRCC1 (AG) and XPD (AC, dominant model) genotypes are strongly associated with increased risk, while XRCC3 shows no significant effect, and APEX1 (GG) is overrepresented in cases, with TT potentially protective. These findings support the hypothesis that impaired DNA repair capacity contributes to disease development, particularly through heterozygous or dominant effects in key repair genes.

REFERENCES

1. Agarwal, A., Mulgund, A., Hamada, A., & Chyatte, M. R. (2015). A unique view on male infertility around the globe. Reproductive Biology and Endocrinology, 13, 37. https://doi.org/10.11 86/s12958-015-0032-1
2. Mascarenhas, M. N., Flaxman, S. R., Boerma, T., Vanderpoel, S., & Stevens, G. A. (2012). National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. PLOS Medicine, 9(12), e1001356. https://doi.org/10.1371/ journal.pmed.1001356
3. Krausz, C., & Riera-Escamilla, A. (2018). Genetics of male infertility. Nature Reviews Urology, 15(6), 369–384. https://doi.org/10.1038/s41585-018-0003-3
4. Krausz, C. (2011). Male infertility: Pathogenesis and clinical diagnosis. Best Practice & Research Clinical Endocrinology & Metabolism, 25(2), 271–285. https://doi.org/10.1016 /j.beem.2010.08.006
5. Barratt, C. L. R., Björndahl, L., De Jonge, C. J., Lamb, D. J., & Osorio Martini, F. (2017). The diagnosis of male infertility: An analysis of the evidence to support the development of global WHO guidance—Challenges and future research opportunities. Human Reproduction Update, 23(6), 660–680. https://doi.org/10.1093/humupd/dmx021

6. Zini, A., & Libman, J. (2006). Sperm DNA damage: Clinical significance in the era of assisted reproduction. CMAJ, 175(5), 495–500. https://doi.org/10.1503/cmaj.060218
7. Aitken, R. J., & De Iuliis, G. N. (2010). On the possible origins of DNA damage in human spermatozoa. Molecular Human Reproduction, 16(1), 3–13. https://doi.org/10.1093 /molehr/gap059
8. Simon, L., Zini, A., Dyachenko, A., Ciampi, A., & Carrell, D. T. (2017). A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Fertility and Sterility, 108(3), 468–483.e5. https://doi.org/10.1016/j.fertnstert.2017.07.019
9. Esteves, S. C., Sánchez-Martín, F., Sánchez-Martín, P., Schneider, D. T., & Gosálvez, J. (2021). Comparison of reproductive outcomes in oligozoospermic men with high sperm DNA fragmentation treated with intracytoplasmic sperm injection or intracytoplasmic morphologically selected sperm injection. Asian Journal of Andrology, 23(4), 408–414. https://doi.org/10.4103/aja.aja_53_20
10. Singh, R., & Jaiswal, D. (2022). Impact of DNA repair gene polymorphisms on male infertility: A review. Reproductive Sciences, 29(2), 349–359. https://doi.org/10.1007/s43032-021-00541-4
11. Lu, J., Wang, Q., Hu, Y., & Wu, W. (2020). Genetic variants in DNA repair pathways and their association with male infertility: A systematic review and meta-analysis. Gene, 754, 144889. https://doi.org/10.1016/j.gene.2020.144889
12. Berwick, M., & Vineis, P. (2000). Markers of DNA repair and susceptibility to cancer in humans: An epidemiologic review. Journal of the National Cancer Institute, 92(11), 874–897. https://doi.org/10.1093/jnci/92.11.874
13. Hoeijmakers JHJ. Genome maintenance mechanisms for preventing cancer. Nature. 2001;411(6835):366–374.
14. Shen, M. R., Jones, I. M., & Mohrenweiser, H. W. (1998). Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Research, 58(4), 604–608.
15. Romanowicz-Makowska H, Smolarz B, Baszczynski J, Samulak D. Polymorphism of the XRCC3 gene and the risk of male infertility in the Polish population. Arch Med Sci. 2012;8(3):499–504. doi:10.5114/aoms.2012.28594
16. Spitz, M. R., Wei, Q., Dong, Q., Amos, C. I., & Wu, X. (2001). Genetic susceptibility to lung cancer: The role of DNA damage and repair. Cancer Epidemiology, Biomarkers & Prevention, 10(8), 839–844.
17. Hu, J. J., Smith, T. R., Miller, M. S., et al. (2001). Genetic regulation of DNA repair and its possible link to cancer risk: A literature review and meta-analysis. Mutation Research, 477(1–2), 1–8. https://doi.org/10.1016/S0027-5107(01)00301-X
18. Abhishek, K., Singh, V., Shukla, V. K., et al. (2019). Role of DNA repair gene polymorphism (APEX1, XRCC1 and XRCC3) in susceptibility to gallbladder cancer in North Indian population. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 843, 13–21. https://doi.org/10.1016/j.mrgentox.2019.05.002
19. Green, M. R., & Sambrook, J. (2012). Molecular Cloning: A Laboratory Manual (4th ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.