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ATM rs189037 polymorphism: A potential genetic risk factor for sperm DNA damage and male infertility

Gülbahar Güzel Erdal[1*], Mahmut Balkan[1], Selahattin Tekeş[1], Diclehan Oral[1], Zuhal Çankiri[2]

1. Institute of Medical Sciences, Department of Medical Biology and Genetics, Dicle University, Turkey
2. Institute of Medical Sciences, Department of Histology and Embryology, Dicle University, Turkey

ABSTRACT

Background: Sperm DNA integrity is critical for fertilization, embryo development, and a healthy pregnancy; DNA damage is an important cause of male infertility. The ATM gene safeguards genomic integrity by orchestrating critical DNA repair pathways. The rs189037 polymorphism in the ATM gene may influence sperm DNA fragmentation (SDF) by affecting this pathway. This study examines the potential link between the ATM rs189037 genetic variant and elevated SDF levels in men diagnosed with idiopathic oligospermia.

Methods: The study involved 40 men with idiopathic infertility and 21 men who were normozoospermic. SDF in semen samples from these 61 participants was analyzed using the TUNEL test. The rs189037 alleles of the ATM gene polymorphisms were genotyped using Taq-Man allele-specific probes in real-time PCR.

Results: This study identified a significant association between genotypic variations of the ATM rs189037 polymorphism and infertility. The genotype frequencies of the rs189037 polymorphism in the patient group were 20% GG, 37.5% GA, and 42.5% AA, whereas in the control group, the respective frequencies were 52.38% GG, 33.33% GA, and 14.28% AA. These findings indicate that the frequency of the A allele was higher in patients than controls ($p<0.05$) and was positively correlated with increased SDF ($p<0.05$).

Conclusions: This study identifies the A allele of ATM rs189037 as a significant genetic factor associated with impaired sperm DNA integrity in idiopathic oligospermic men, suggesting its potential utility as a novel biomarker for the diagnosis and subclassification of idiopathic male infertility.

Keywords: ATM, oligospermic infertile men, rs189037, sperm DFI, TUNEL

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INTRODUCTION

Male reproductive dysfunction is a multifactorial pathological condition, the etiology of which is associated with various genetic abnormalities [1]. This disorder exhibits marked clinical heterogeneity, presenting along a spectrum that ranges from complete testicular sperm absence (azoospermia) at one extreme to subtle impairments in sperm quality at the other [2]. Molecular diagnostics, such as Y chromosome microdeletion screening and monogenic disorder analysis, enable the precise evaluation of male infertility [3]. However, despite these advances, definitive genetic causes are identified in only approximately 4% of infertile men, while the etiology remains elusive in 60–70% of cases, underscoring the need for expanded genetic investigations [4]. Among the various clinical presentations of male infertility, oligospermia—defined as a sperm concentration below 15 million/mL in the ejaculate—represents one of the most prevalent forms of impaired spermatogenesis [5]. The development and progression of this disorder commonly result from genetic alterations [3], revealing the intricate polygenic nature of male reproductive impairment. In this context, contemporary andrological research has increasingly focused on elucidating the mechanisms behind unexplained oligospermia, particularly its association with defective spermatogenic processes [6–8]. Mounting experimental and clinical data implicate compromised DNA repair mechanisms, influenced by both heritable genetic variations and acquired epigenetic changes, as a critical factor in the pathogenesis of spermatogenic dysfunction [9].

In mammals, double-strand breaks (DSBs) that occur during spermatogenesis can be induced by various exogenous sources, including ionizing radiation, genotoxic compounds, as well as endogenously generated reactive oxygen species (ROS), which are natural by-products of

* Corresponding author: Gülbahar Güzel Erdal, Institute of Medical Sciences, Department of Medical Biology and Genetics, Dicle University, Turkey. E-mail: gulbaharguzel@yahoo.com

oxidative phosphorylation and metabolic activity. Additionally, DSBs may arise during meiotic recombination or as a consequence of DNA repair processes. The impact of these DSBs can be extensive, often resulting in a multitude of cellular consequences such as cell cycle arrest, alterations in gene expression, apoptosis, and failure in spermatogenesis [10]. The nature and severity of the damage sustained determine the specific cellular consequences observed.

Proper DSB repair is biologically indispensable, and cells have evolved two complementary systems to maintain genomic integrity: HR (homologous recombination), which ensures genetic stability through homologous pairing, and NHEJ (non-homologous end joining), which rapidly restructures DNA ends despite potential mutagenic consequences [11]. The mechanism selected for repair depends on the phase of the cell cycle or the availability of specific components of both mechanisms [12]. HR predominates during the S and G2 phases, utilizing the intact sister chromatid as a replication template, thus contributing to genomic fidelity. This repair mechanism emphasizes the essential role of HR in maintaining genetic stability.

The ataxia-telangiectasia mutated (ATM) kinase serves as the master regulator of HR-mediated DSB repair, orchestrating checkpoint activation and repair factor recruitment [13]. ATM, located on chromosome 11q23, encodes a critical PI3K-like kinase that coordinates the DNA damage response. In Ataxia-Telangiectasia (AT) patients, biallelic ATM mutations impair DSB repair mechanisms, leading to the disease triad of 1) progressive ataxia, 2) telangiectasias, and 3) cancer susceptibility. ATM's role in activating cell cycle arrest prevents the proliferation of unrepaired DNA lesions, which explains the radiosensitivity and chromosomal aberrations observed in AT [14,15]. Furthermore, ATM deficiency is manifested by distinct reproductive pathologies between the sexes: ovarian failure in women (amenorrhea and anovulation) and spermatogenic arrest in men, highlighting the crucial role of ATM in human gametogenesis [16]. Studies in male mice with ATM protein kinase deficiency (ATM-/-) have revealed the critical role of this enzyme in the early stages of spermatogenesis. Loss of ATM function leads to severe premeiotic germ cell abnormalities, characterized by progressive chromosomal instability, apoptotic depletion of spermatogenic cells, and ultimately advanced-stage testicular degeneration [17-20].

In recent years, single-nucleotide polymorphisms (SNPs) have emerged as one of the most important molecular markers used to diagnose and profile the genetic risk of male infertility. In this context, the rs189037 G>A polymorphism has been proposed to be involved in the etiology of human INOA (idiopathic non-obstructive azoospermia) by affecting ATM expression. The rs189037 G>A polymorphism is a functional G>A variant located within the E2F1 binding motif in the ATM promoter region. This polymorphism induces structural rearrangements in the ATM promoter, altering the kinetics of transcription-initiation complex formation, thereby influencing the expression of the ATM gene [21]. This study aims to molecularly characterize the association between the rs189037 polymorphism and SDF in oligospermic men.

METHODS

Selection of study subjects

In this study, two prospectively defined groups were carefully established. The first group consisted of 40 oligozoospermic patients meeting World Health Organization (WHO) diagnostic criteria, and the second group consisted of 21 normozoospermic men with confirmed fertility. Both groups were appropriately matched for age and other demographic characteristics. Oligozoospermic individuals were selected from patients diagnosed with primary infertility at the Assisted Reproduction Center of the Dicle University Medical Faculty, whereas control participants were selected from the same geographic region to minimize potential environmental and socioeconomic confounders. All participants underwent a comprehensive evaluation protocol that included physical examination, standardised semen analysis performed twice according to WHO 2021 guidelines, assessment of serum reproductive hormone levels (FSH, LH, and total testosterone), testicular ultrasonography, cytogenetic analysis, Y-chromosome microdeletion screening, and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay for evaluation of SDF. Inclusion criteria for the oligozoospermic group were as follows: 1) documented history of primary infertility, 2) sperm concentration below 15 million/mL with normal motility and morphology, 3) normal external genital anatomy on physical examination, and 4) age between 20 and 45 years. This comprehensive and standardized diagnostic approach ensured the accurate phenotyping of participants and maintained methodological consistency with current standards in andrology research.

Approval for the research protocol was received from the Dicle University Faculty of Medicine Non-Interventional Research Ethics Committee (2023/179, date: 14 June 2023). Written informed consent was obtained from all participants prior to their inclusion in the study.

TUNEL assay

This case-control study analyzed sperm DNA fragmentation (SDF) in semen samples from 40 infertile men and 26 fertile controls, with all participants providing ethics-approved informed consent. Seminal plasma was separated by centrifugation (500 × g, 7 min), and the cellular pellet was washed with PBS, fixed in 4% PFA, and stored at 4°C. Fixed samples were processed on poly-L-lysine-coated slides, permeabilized with 0.1% sodium citrate/0.1% Triton X-100, and subjected to a TUNEL assay (Roche Diagnostics GmbH) following established protocols [22,23]. After enzyme labelling (37°C, 1 h) and PBS washing, dual fluorescence microscopy (Olympus BX51, 40×) using DAPI (461 nm) and FITC (519 nm) enabled simultaneous nuclear identification and fragmentation detection. The DNA fragmentation index (DFI) was calculated from ≥500 spermatozoa per sample (FITC-positive/DAPI-positive × 100) using ImageJ software, with representative results illustrated in Figure 1. This standardized approach ensured reliable quantification of SDF while maintaining experimental consistency across all samples.

Molecular analysis of ATM rs189037 polymorphism

High-quality genomic DNA was isolated from freshly collected peripheral blood mononuclear cells using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany) on an automated QIAcube HT platform, ensuring consistent yields of pure, high-molecular-weight DNA suitable for downstream applications. Subsequent fluorometric quantification with the Qubit™ 4 system (Invitrogen, USA) confirmed optimal DNA concentrations (20–50 ng/µL) prior to genotyping analysis. TaqMan allelic discrimination assays (Thermo Fisher, USA) were then performed in 25 µL reactions containing 2 µL of template DNA under standardized cycling conditions (95°C for 10 min, followed by 40 cycles of 95°C for 10 sec and 60°C for 1 min) to ensure reliable SNP detection for genetic studies.

For the analysis of variant 189037, the primers 5′-GCTGCTTGGCGTTGCTTC-3′ (forward) and 5′-CATGAGATTGGCGGTCTGG-3′ (reverse) were used. The sequence context CTAACGGAGAAAAGAAGCCGTGGCC[A/G]CGGGAGGAGGCGAGAGGAGTCGGGA was examined for the presence of nucleotide variations.

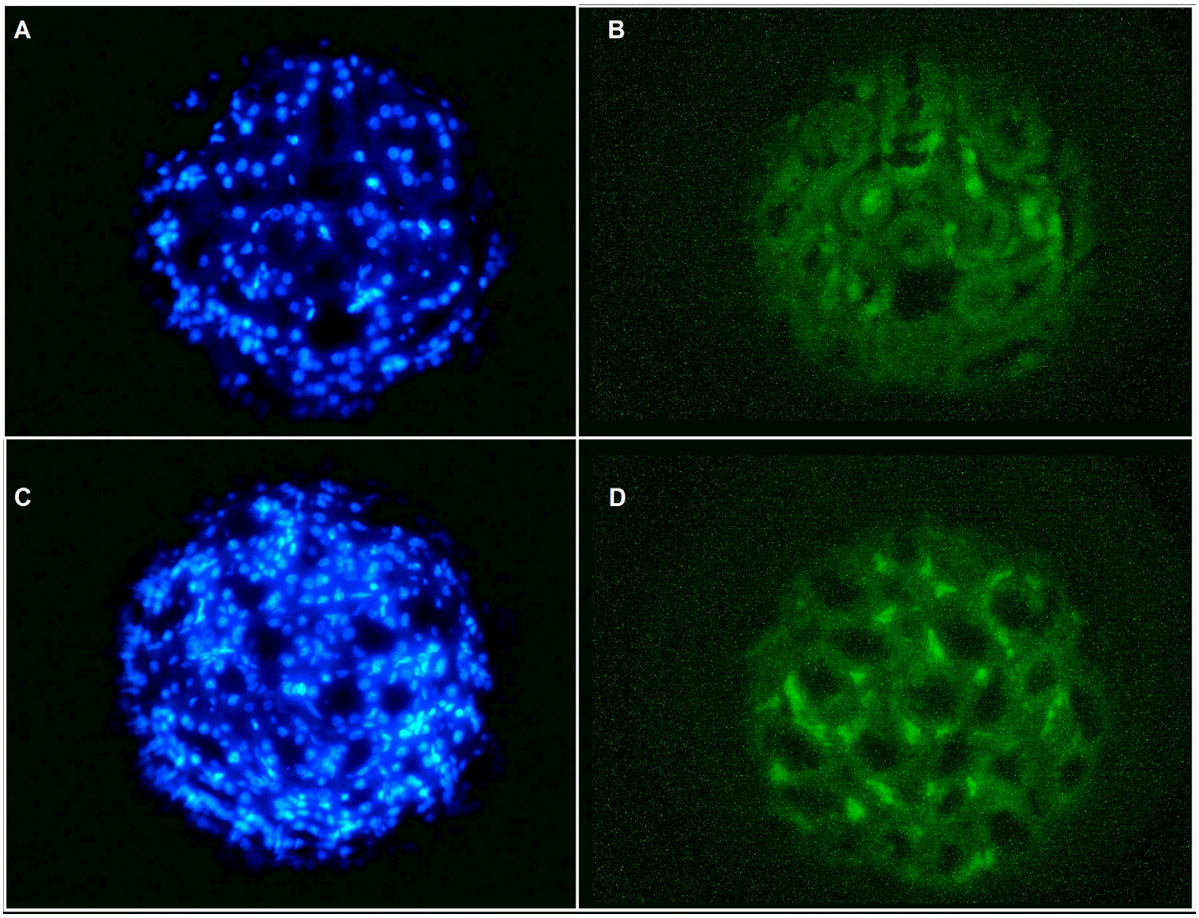

Figure 1. Fluorescence microscopy images of sperm samples stained with DAPI (461 nm, blue) and FITC (519 nm, green) for the assessment of sperm DNA fragmentation using the TUNEL assay

Panels A/B and C/D are paired. A: DAPI-stained image of a sperm sample with low DNA fragmentation. B: Corresponding FITC-stained image of the same sperm sample showing minimal TUNEL-positive cells. C: DAPI-stained image of a sperm sample with high DNA fragmentation. D: Corresponding FITC-stained image of the same sperm sample showing numerous TUNEL-positive cells indicating extensive DNA fragmentation.

Statistical analysis

A comparison of allele and genotype frequencies between the two groups was performed using Pearson's chi-square statistical test. Hardy-Weinberg equilibrium calculations were conducted to evaluate the expected genotype distributions. In cases where sample sizes were limited, Fisher's exact test was employed. Parametric continuous variables were analyzed using the independent samples t-test, while non-parametric variables were evaluated using the Mann-Whitney U statistical test. Intervariable relationships were assessed using Spearman's rank-order correlation (ρ) test. Diagnostic cut-off values were determined through receiver operating characteristic (ROC) curve analysis. Statistical analyses were conducted using SPSS version 11.5 (SPSS Inc., Chicago, IL), with a two-tailed significance threshold set at $p<0.05$.

RESULTS

Demographic and clinical profiles of the cohort

The present case-control study included 40 men diagnosed with idiopathic oligospermia (cases) and 21 fertile men with normozoospermia (healthy controls). The mean ages of the two groups were comparable (controls: 33.43 ± 1.11 years; cases: 31.65 ± 0.80 years; $p=0.811$), confirming appropriate age matching. A systematic comparison was conducted between the groups concerning semen parameters and SDF levels. A comprehensive summary of the laboratory findings is presented in Table 1.

Sperm DFI and its association with semen parameters and genetic polymorphism

Quantitative analysis revealed significantly elevated DFI levels in oligospermic patients compared to normozoospermic controls (Table 2). DFI demonstrated strong negative correlations with conventional semen parameters, including sperm concentration, total sperm count, progressive motility, and normal morphology (Table 2). Notably, a robust positive correlation was observed between DFI and the rs189037 polymorphism , suggesting a potential genetic influence on sperm DNA integrity. The discriminatory capacity of SDF between patients and controls was assessed using ROC curve analysis, yielding an area under the curve (AUC) of 0.971 ($p<0.0001$). The optimal DFI cut-off value distinguishing patients from controls was calculated as 54.63%, with a sensitivity of 95% and a specificity of 86% (Figure 2).

The role of rs189037 (G>A) in oligospermia: A candidate gene approach

Genotyping of the rs189037 (G>A) polymorphism was performed using real-time PCR with allele-specific probes. The allele and genotype distributions in both groups conformed to Hardy-Weinberg equilibrium (HWE; p>0.05) (Table 3). Comparative analysis revealed statistically significant differences in allele and genotype frequencies between oligospermic patients and controls (p<0.05). Notably, the A allele of rs189037 was significantly associated with an increased risk of oligospermia (OR [95% CI]; p<0.05). In the genotype-based analysis, the homozygous AA genotype emerged as a strong risk factor for oligospermia (p<0.05), whereas the heterozygous GA genotype showed no significant association (p>0.05).

Table 1. Semen parameters of infertile and control groups

	OLIGOSPERMIA			CONTROL			*p* value
	n	Mean ± SD	Median (range)	n	Mean ± SD	Median (range)	
Sperm concentration	40	9.72 ± 0.45	9.70 (2.17-14.60)	21	122.30 ± 13.46	124.10 (20.20-296.20)	< 0.0001
NonProgressive motile spermatozoa	40	60.43 ± 3.48	67.00 (10.00-99.00)	21	37.30 ± 4.40	29.00 (16.00-76.00)	< 0.0001
Total motile sperm	40	13.82 ± 1.76	13.75 (0.20-53.80)	21	93.70 ± 17.50	71 (10.11-362.60)	< 0.0001
Total progressive motile sperm	40	8.92 ± 1.32	6.85 (0.00-27.14)	21	81.14 ± 15.48	67.00 (7.90-331.20)	< 0.0001
Normal sperm morphology.	40	3.28 ± 0.59	2.90 (0.00-21.30)	21	47.49 ± 5.93	44.70 (1.10-89.00)	< 0.0001
Sperm DNA fragmentation	40	77.43 ± 2.15	78.85 (41.26-98.20)	21	24.17 ± 4.59	12.50 (5.40-65.50)	< 0.0001

Table 2. Comparison of rs189037(G>A), DNA fragmentation index (DFI), and semen parameters

		Age	rs189037	Sperm concentration	Total sperm count	Total progressive motile sperm count	Normal morphology
DFI	r	0.212	0.754	–0.834	–0.701	–0.711	–0.781
	p value	0.101	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	N	61	61	61	61	61	61

Table 3. Genotype / allele frequencies of rs189037 (G>A)

Polymorphisms Genotypes	Oligospermia (N=40) n (%)	Control (N=21) n (%)	*p* value	X^2	Odds Ratio	%95 CI
GG	8 (20.0%)	11 (52.4%))		Reference		
GA	15 (37.5%)	7 (33.3%)	0.093	2.815	2.946	0.821-3.217
AA	17 (42.5%)	3 (14.3%)	0.014	6.038	7.792	1.690-35.925
GA+AA	32 (80.0%)	10 (47.6%)	0.020	5.307	4.400	1.386-13.963
Allele frequency						
G	31	29		Reference		
A	49	13	0.002	8.939	3.520	1.594-7.800

DISCUSSION

Male infertility, affecting over 50 million men worldwide [24,25], is a multifactorial health problem in which sperm DNA integrity plays a decisive role in fertility outcomes [26]. DNA double-strand breaks (DSBs), which frequently occur during the mitotic and meiotic divisions of spermatogenesis, pose a serious threat to genomic stability [27]. ATM kinase, a key molecule regulating DSB repair pathways, meiotic recombination, and cell cycle checkpoints [17,28,29], plays a decisive role in spermatogenesis by maintaining genomic stability in male germ cells [30–33]. Previous studies have demonstrated that ATM deficiency in mice leads to severe testicular degeneration and spermatogenic arrest [17,30–32], while its activation in response to DNA damage promotes autophagy and cell cycle control in male germ cells [33,34]. Moreover, alterations in ATM function have been associated with endocrine abnormalities and infertility-related pathologies in human cohorts [16,35]. Given the limitations of conventional semen analysis in reflecting genomic integrity, we further evaluated SDF to gain deeper insights into the molecular mechanisms underlying male infertility.

In this study, the relationship between the rs189037 polymorphism in the ATM gene and SDF was investigated. The effects on male fertility of this gene variant, a critical component of the DSB repair mechanism, were evaluated. Our findings revealed a significant deterioration in semen parameters — including concentration, motility, morphology, and viability — in the patient group compared to the control group, based on World Health Organization (WHO) standards ($p<0.0001$). These results are consistent with the findings of Agarwal et al. [36], who reported normal semen parameters in fertile men and abnormal values in oligospermic infertile men. However, semen analysis alone is limited to the assessment of specific critical parameters of sperm functionality and does not provide direct information about sperm DNA integrity. Therefore, the evaluation of SDF has emerged as an important parameter for a more comprehensive assessment of male fertility. The latest version of the WHO Sperm Analysis Manual also recommends the assessment of SDF among defined patient populations in clinical settings [37]. In our study, we employed the TUNEL assay, a highly sensitive and specific method distinguished by its capacity to detect actual DNA damage [38-41].

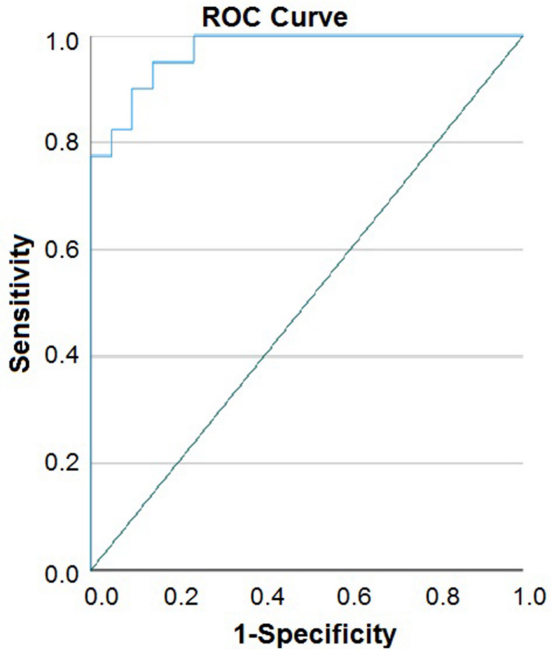

Figure 2. ROC curve illustrating the diagnostic performance of sperm DNA fragmentation (SDF)

Upon examining DFI values, we found that the DFI rate was notably high at 78.85% in the patient group, while it was 12.5% in the control group. This difference was statistically significant ($p<0.0001$) and aligns with numerous findings in the literature. Sergerie et al. reported a DFI rate of 92.8% in infertile men, while Sharma et al. identified DFI rates of 28.5% in infertile men and 11.9% in fertile men, demonstrating a strong association between DFI and infertility [39,42]. Additionally, a large-scale meta-analysis covering 28 studies also supported that DFI rates in infertile men were significantly higher compared to control groups ($p< 0.001$) [43]. When evaluating the relationship between DFI values and semen parameters, our findings revealed a positive correlation between higher DFI rates and abnormal sperm morphology incidence, and a negative correlation with progressive sperm motility, total motility, and total sperm concentration. These findings are consistent with the results of Gu et al. [44] and Ebrahimi et al. [45] in studies conducted on infertile men.

Disruption of DNA repair mechanisms can increase the susceptibility of germ cells to apoptosis, causing significant problems in the spermatogenesis process. Particularly, the ATM kinase is one of the most crucial molecules responsible for DSB repair during meiotic recombination [28]. The rs189037 polymorphism analyzed in our study is located in the promoter region of the ATM gene and is a significant genetic variant that can influence transcriptional regulation [46,47]. Functional data indicate that this variant reduces the binding affinity of the E2F1 transcription factor, consequently decreasing ATM gene expression and adversely affecting DNA repair capacity [48,49].

According to our results, the A allele significantly increases the risk of oligospermia, with the homozygous AA genotype being more frequently observed in the patient group ($p<0.05$). No significant increase in risk was detected among heterozygous GA genotype carriers ($p>0.05$). This finding supports the potential role of the ATM rs189037 polymorphism as a genetic marker in the development of oligospermia. While studies investigating the relationship between this polymorphism and infertility remain limited, available findings show inconsistency. Our results contradict the findings of Ji et al., who found no significant association in infertile men [50], but are partially consistent with those of Li et al., who reported a nearly two-fold increase in risk for the AA genotype among azoospermic patients [21]. Furthermore, the rs189037 polymorphism not only affects ATM gene expression but also functions as a cis-regulatory variant, demonstrating a cis-eQTL (expression quantitative trait locus) effect at this locus. In A allele carriers, transcriptional downregulation has been observed in ATM and neighboring genes, resulting in decreased mRNA levels [51]. Mechanistically, the A allele is thought to disrupt transcription factor binding sites or trigger epigenetic processes such as promoter CpG methylation, repressive histone modifications (H3K27me3), and reduced chromatin accessibility. Notably, in malignancies such as colorectal and prostate cancer, the rs189037 variant has been associated with methylation at four specific CpG regions within the ATM promoter, leading to a 25–40% reduction in ATM expression [52,53].

The ATM gene is notable not only for its relationship with infertility but also as a genetic risk factor for certain malignancies, particularly prostate cancer. Genome-wide studies have shown that pathogenic variants in the ATM gene increase the risk of prostate cancer development by 2- to 5-fold [54]. A study conducted in Poland identified mutations in the ATM gene in a subset of individuals with a familial history of prostate cancer, which were significantly associated with more aggressive disease phenotypes [55].

These findings suggest that the ATM gene may play a pivotal role in both germ cell biology and the etiopathogenesis of certain solid tumors, notably prostate cancer. In conclusion, our data indicate that the ATM rs189037 polymorphism may play a significant role in the risk of oligospermia, likely mediated via epigenetic regulation. To test this hypothesis, advanced epigenetic and functional studies investigating the relationship between the rs189037 genotype and ATM promoter methylation status in infertile men are warranted. Furthermore, the functional role of the ATM gene in spermatogenesis and its relationship with DNA repair mechanisms remains of crucial importance for understanding male infertility and developing novel treatment strategies.

Study limitations and future perspectives

This study provides compelling evidence linking the ATM rs189037 polymorphism to male infertility and sperm DNA damage; however, several limitations should be acknowledged. The single-center design and relatively modest sample size may limit the generalizability of the findings, particularly given potential population-specific genetic differences. Future multicenter studies involving larger, ethnically diverse cohorts are necessary to confirm these associations. Although the TUNEL assay reliably detects DNA strand breaks, it does not assess oxidative base damage or chromatin integrity, highlighting the need for complementary methodologies such as the SCSA and Comet assays in subsequent research. Additionally, the functional implications of the A allele remain unclear, as epigenetic and expression analyses were beyond the scope of this study. Integrating methylation profiling, transcriptomic data, and protein expression analyses would substantially enhance mechanistic understanding. Moreover, unmeasured environmental factors, including lifestyle habits and toxic exposures, represent potential confounders that should be systematically addressed in future investigations.

CONCLUSIONS

This study demonstrated that the ATM rs189037 polymorphism may be a significant genetic risk factor for male infertility, particularly in the development of oligospermia. The presence of the A allele appears to increase susceptibility, potentially by impairing DNA repair capacity and through epigenetic regulatory mechanisms. Additionally, infertile men exhibited significantly elevated sperm DNA fragmentation rates, and higher DNA Fragmentation Index (DFI) values were associated with adverse effects on semen parameters, including sperm morphology, motility, and concentration. These findings underscore the pivotal role of sperm DNA integrity in male infertility and support the integration of DNA damage assessments alongside conventional semen analyses in clinical practice.

ABBREVIATIONS

AT – Ataxia-Telangiectasia

ATM – Ataxia-telangiectasia mutated

cis-eQTL – expression quantitative trait locus

DFI – Sperm DNA fragmentation index

DSB – Double Strand Breaks

HR – homologous recombination

INOA – idiopathic nonobstructive azoospermia

NHEJ – non-homologous end joining

ROC – receiver operating characteristic

ROS – Reactive Oxygen Species

SDF – Sperm DNA fragmentation

SNP – single-nucleotide polymorphism

TUNEL – Terminal deoxynucleotidyl transferase dUTP nick end labeling

WHO – World Health Organization

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AUTHORS' CONTRIBUTION

GGE – conceptualization, study design, methodology development, validation, formal analysis, data curation, investigation, visualization, original draft preparation, and overall research execution quality control, and final approval of the manuscript

MB – validation of results, original draft preparation, writing – review & editing, and data interpretation

ST – methodology optimization, validation procedures, project administration, resources acquisition, and funding procurement

DO – methods optimization, procedures and results validation

ZB – project supervision, writing – review & editing, critical intellectual input, quality control the manuscript

All authors have read and agreed to the published version of the manuscript

CONFLICT OF INTEREST

None to declare.

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