



Men with genetic predisposition face greater fertility challenges when exposed to electromagnetic radiation

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Abstract

Purpose This study investigates the synergistic effects of genetic variants in core meiotic regulator genes—**SPO11**, **RNF212**, and **SYCP3**—and co-occurring exposure to electronic radiation as risk factors for azoospermia among Bengali-speaking men from West Bengal, India.

Materials and methods A total of 708 azoospermic individuals, who tested negative for Y chromosome microdeletions, and 640 healthy controls were genotyped using Sanger's dideoxy sequencing. Genetic variants identified included SPO11 rs3764674T>G, MN650122delA, MN708963insA, MN708964insA, MN720359T>A; RNF212 MN737491delA, rs4045481C>T; and SYCP3 rs10860779C>A, MN901901delA, and MN995822delA. Binary logistic regression was used to assess the interaction between genetic variants, electronic radiation exposure, and age, with fertility status as the outcome variable.

Results The analysis revealed a significant association between genetic variations in meiotic regulators and increased risk of azoospermia, particularly among men aged 30 years or older who were exposed to electronic radiation. The findings suggest that effect of electronic radiation may exacerbate meiotic errors, impair germ cell development, and further reduce fertility in affected individuals.

Conclusions Men carrying genetic variations in SPO11, RNF212, and SYCP3 and exposed to electronic radiation are at an elevated risk of azoospermia, particularly with advancing age. This information may be considered in routine screening of assisted reproductive technology (ART) practices, which will enable clinicians to tailor management strategies for male infertility.

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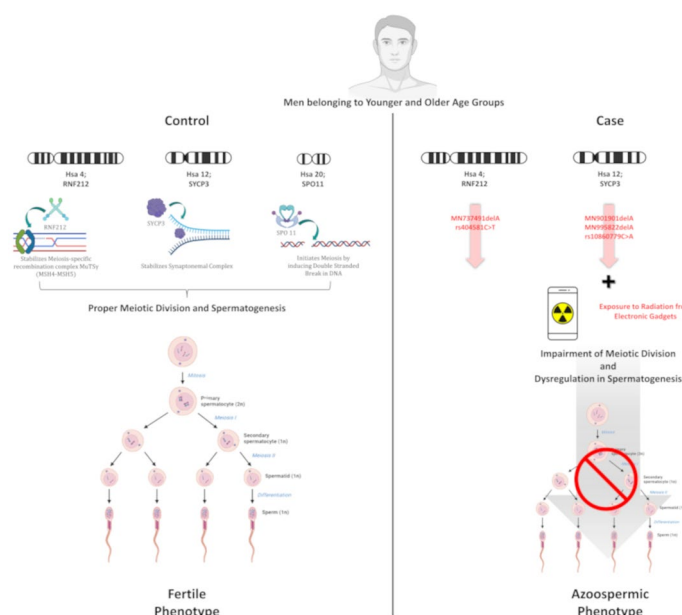
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Graphical Abstract



Interactions among the variants of SPO11, RNF212, SYCP3, age and radiation exposure that impair natural spermatogenesis process

Keywords Azoospermia · Environmental genomics · Genetic variation · Meiosis · Radiation effects

Abbreviations

ART	Assisted Reproductive Technology
GxE	Gene × Environment interaction
SCT	Smokeless Chewing Tobacco
MRV+	Meiotic Regulator Variant–Positive
WT	Wild Type
and MUT	Mutant Genotype
NMD	Splice junction/Intron/NMD variant

Introduction

Spermatogenesis is a finely tuned process and regulated by complex interplay of genes [1]. Any alteration in the core meiotic regulators in spermatogenesis can affect the production of male gametes. Male factor infertility is implicated in approximately 50% of all diagnosed infertility cases [2]. Male infertility often appears as idiopathic, with its underlying causes remaining unclear even after extensive evaluation. Autosomal gene polymorphisms [3, 4] and chromosomal copy number variation [5] have also been identified among idiopathic cases with poor sperm quality [6–8].

Three spermatogenic regulators namely *SPO11* (SPO11 initiator of meiotic double-strand breaks, also known as TOPVIA/TOPOVIA) gene (OMIM ID 605114, location 20q13.31), RNF212 (ring finger protein 212) (OMIM ID

612041, location 4p16.3) and SYCP3 (OMIM ID 604759, location 12q23.2) have been characterized previously for their mutations or polymorphisms and their implication in male sterility. The SPO11 creates double-strand breaks (DSB) to initiate meiosis, in both yeast and human [9, 10]. In mice, SPO11 deficiency is known to cause spermatogonial arrest and its SNPs were identified among clinical cases of male infertility [11]. The RNF212, a ubiquitin ligase, facilitates synaptonemal complex formation in meiosis [12]. Studies revealed positive association with RNF212 mutations with the incidence of azoospermia [13]. The SYCP3 is a component of synaptonemal complex [14, 15] and mutations of this gene are known to cause azoospermia [16, 17].

Beside genetic risk factors, epidemiological or habitual factors are also known to increase risk of infertility in both men and women [18–23]. The adverse effects of smokeless chewing tobacco (SCT), cigarettes, alcohol, drug dependence, exposure to harmful chemicals and radiation are reported risk factors for human spermatogenesis impairment [24–31]. It is still unclear if radiation from mainstream electronic devices like laptops and mobile or cell phones essentially increase the risk of spermatogenesis. It is of utmost interest to determine whether exposure to electronic radiation interacts with other risk factors when they co-occur or exerts any synergistic effects with genetic predispositions that could impair optimal spermatogenesis. Researchers

have demonstrated that environmental or epidemiological factors can aggravate the symptoms of many neurological illnesses with vulnerable genetic backgrounds [32, 33]. Here, we have designed the gene-environment interaction (gene $\times$ environment) model to characterize the synergistic effects of genetic variations and exposure to electronic radiation on male fertility. We conducted analyses to answer two key questions (1) do genetic variations of SPO11, RNF212 and SYCP3 elevate the risk of azoospermia among Bengali men? (2) How does electronic radiation exposure impart additional risk of azoospermia when co-occurs with genetic variations in the meiotic regulators? In this context, we applied a **Gene $\times$ Environment (G $\times$ E) interaction model**, wherein we assessed the combined influence of genetic susceptibility (variants in SPO11, RNF212, SYCP3) and environmental exposure (non-ionizing radiation) on the risk of azoospermia. This model enables understanding how environmental exposures may amplify the effects of underlying genetic predispositions.

Materials and methods

Ethics declarations

This study was reviewed and approved by the **Institutional Human Ethics Committee, University of Calcutta**, West Bengal, India (**Approval No. CU/BIOETHICS/HUMAN/2306/3044**, dated 04/12/2017). All procedures were conducted in accordance with the principles of the **Declaration of Helsinki** and guidelines by the **Indian Council of Medical Research (ICMR)**.

Consent statement

All the samples were collected with informed written consent from the volunteers.

Sample cohort

The study included 708 azoospermic and 640 proven fertile males recruited from the Institute of Reproductive Medicine (IRM) in Kolkata. Non-obstructive azoospermia was confirmed via semen analysis, indicating a complete absence of sperm due to impaired spermatogenesis rather than any physical blockage, while controls were fertile males with normal seminograms and no fertility issues. Demographic similarity and homogeneity of ethnicity were maintained. Exclusion criteria included poor health, infectious diseases, testicular abnormalities, anabolic steroid use, and insufficient epidemiological data. Control individuals had fathered at least one child within the past 5 years, and their partners

had no known reproductive complications. Controls were selected based on self-reported daily exposure to electronic radiation not exceeding 1 h, and residing at distances greater than 2 m from active sources such as Wi-Fi routers or mobile devices during sleep. This threshold approximates a whole-body exposure below 0.08 W/kg (Specific Absorption Rate), consistent with international safety guidelines outlined by IEEE (Institute of Electrical and Electronics Engineers) C95.1-2019 and ICNIRP (International Commission on Non-Ionizing Radiation Protection), which recommend maximum SAR limits of 0.08 W/kg for the general public. Confidentiality of personal data was maintained using individual codes, and diagnosis of azoospermia was based on at least two semen analyses conducted on separate occasions following WHO guidelines 6th edition (2021). In selected cases with clinical ambiguity, serum hormone levels (FSH, LH, testosterone) and testicular ultrasound were also evaluated. We selected case subjects those who reported use a laptop (electromagnetic field of 4000 MHz) on their lap for at least four hours daily or keep a mobile phone in their trouser pocket for a similar duration, (electromagnetic field of 2000 MHz), or both. The radiation exposure in this study refers to **non-ionizing electromagnetic radiation**, primarily in the radiofrequency (RF) range, emitted by commonly used electronic devices such as mobile phones (900–2450 MHz) and laptops (400 to 800 THz optical). These devices do not emit ionizing radiation but may generate localized thermal and oxidative stress in tissues, as reported in prior clinical and preclinical studies. In select azoospermic patients, empirical hormone therapy (FSH and hCG) was administered as part of standard clinical management to stimulate spermatogenesis. Treatment duration, dosage, and responsiveness were documented based on sperm parameters and hormonal reassessment after 90 days.

Clinical evaluation

To comprehensively characterize the study participants, all azoospermic individuals underwent detailed clinical evaluation including hormonal profiling (FSH, LH, testosterone), scrotal ultrasonography, and semen analysis. Karyotyping was performed to negate gross chromosomal anomalies, and Y chromosome microdeletion analysis (AZF regions) was conducted to detect known causes of non-obstructive azoospermia. In cases where obstructive azoospermia was suspected based on clinical history or imaging findings, targeted **CFTR** gene analysis was additionally performed. This multi-tiered diagnostic approach ensured differentiation between obstructive and non-obstructive etiologies of azoospermia, allowing for accurate classification of the participants. Additionally, each participant's medical history was recorded to assess the presence of systemic conditions

known to affect fertility, including diabetes mellitus, hypertension, thyroid dysfunction, and autoimmune disorders. Standard biochemical assays were performed to evaluate relevant endocrine parameters such as serum FSH, LH, testosterone, and prolactin levels. This multi-tiered clinical profiling helped rule out confounding causes of azoospermia and ensured a homogenous idiopathic case cohort for the genetic and environmental risk analysis.

Tissue collection

Nearly 2 ml of venous blood samples were collected in EDTA-coated vacutainer tubes, from all participants only after obtaining full written informed consent, in accordance with institutional ethical guidelines and approval. Due to ethical and procedural limitations associated with invasive sampling in a non-clinical setting, testicular biopsies could not be performed. Following the guidelines of World Health Organization (WHO), semen samples were collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence for testing and analysis [34]. The semen samples are tested at the IRM laboratory in Kolkata to classify them qualitatively and quantitatively. According to the definition by World Health Organization, the term ‘azoospermia’ suggests no sperm in collected semen sample (i.e., no pellets of any kind).

DNA isolation

Genomic DNA was extracted from all blood samples using the QIAamp Blood Mini Kit from QIAGEN (Hilden, Germany) and tested subjectively and quantitatively on 0.8% agarose gel.

Microdeletion study

To rule out any potential Y chromosomal microdeletions, we have cross-checked the azoospermic samples with Panels of STS markers covering the length of the Y chromosome (for AZFa -SY121, SY182, DFFRY, SY86, DBY, SY83; for AZFb -SY34, SY124, SY130, RBM-1, RBM-2, SY143, SY134, SY127 and for AZFc- BPY2, SY254, SY255, SY239, SY242, SY1197).

Genotyping

Sanger's dideoxy sequencing was done using Primer3 program (<https://primer3.ut.ee/>) designed IDT ‘forward’ and ‘reverse’ primers for SPO11 (transcript ID: SPO11-201, ENST00000345868.8, CCDS13456), RNF212 (transcript ID: RNF212-203, ENST00000433731.7, CCDS46996) and SYCP3 (transcript ID: SYCP3-202, ENST00000392924.2,

CCDS9087) reading frames. The Primer3 program (<https://primer3.ut.ee/>) was used to design primers. The PCR results were examined using an ABI Prism 377 DNA sequencer followed by analyses of result. with FinchTV software. Results (forward and reverse strands) were compared to reference sequences using NCBI BLAST and EMBOSS NEEDLE.

Statistical analysis

Pearson's χ^2 test was employed to compare allele and genotype distributions between case and control groups and to verify whether allele frequencies conformed to Hardy–Weinberg equilibrium. Fisher's exact test was used to calculate the odds ratios along with their corresponding 95% confidence intervals (CI). All statistical analyses were performed using the GraphPad Instat program (Graphpad Instat software, SanDiego, CA), with a significance level of 95%. To verify the reliability of the statistical test, we calculated the power of a two-tailed Fisher exact test for two independent proportions (Small sample test) [35]. G*power [36] software was used to calculate the statistical interpretation's power (1- β). In the present analysis, we employed **binary logistic regression models** that included multiplicative interaction terms to assess whether the effect of genetic variants on azoospermia risk was modified by environmental radiation exposure and age. These interaction terms (e.g., Genotype $\times$ Radiation, Genotype $\times$ Age) allowed us to explore **gene-environment (Gx E) synergy**, rather than isolated main effects alone. Azoospermia was treated as the dependent outcome, with exposure to electronic radiation and age as independent predictors.

Results

Among the 640 control individuals, 74 (11.56%) were found to carry polymorphic variants in at least one of the three genes—SPO11, RNF212, or SYCP3. Importantly, none of the novel InDel variants (e.g., SPO11 MN650122delA, MN708963insA, MN708964insA; RNF212 MN737491delA; SYCP3 MN901901delA, MN995822delA) were observed in the control group. The variants detected in controls were limited to three previously reported SNPs: SPO11 rs3764674 T>G ($n=34$), RNF212 rs4045481 C>T ($n=2$), and SYCP3 rs10860779 C>A ($n=38$) (Supplementary Table S1). No individuals in the control group had multiple co-occurring mutations across the three genes. This supports the finding that novel variants identified in the case cohort are likely to be pathogenic or disease-associated, while the rare presence of common SNPs in controls may represent low-penetrance or benign polymorphisms.

Stratification of sample population

The age of participants ranged from 18 to 50 years, with the average being 30.4 ± 5.5 years (mean $\pm$ SD). The case group has been stratified into two categories, namely young ($N=262$; age ≤ 30 years) and older ($N=446$; age >30 years). Among the young men 174 were tested positive for Meiotic Regulator Variant (MRV+) (any one of three genes or more than one;) and 88 reported wild type (MRV-). Among the older men 327 were found MRV+ and 119 had MRV-. Similarly, within the Control group, 246 individuals were from young group with 23 in MRV+ and 223 in MRV- categories. The control older age group included 51 MRV+ and 343 MRV- cases. Further stratification was done on the basis whether or not the subjects were exposed to electromagnetic radiation from laptop or mobile phone kept in their trouser pocket.

Analysis of genetic variants

We found ten genetic variants from SPO11, RNF212, and SYCP3 reading frames in our study cohort (following NCBI database), of them seven were classified as ‘novel’, and three were previously known tagged SNPs. Out of these ten variants, four are single nucleotide changes, four deletions, and two insertions (InDel). Detail of variants and their accession no. are available online at <https://www.ncbi.nlm.nih.gov/nuccore/>.

Association of meiotic regulator variants with azoospermia

We determined the frequency of all ten genetic variants, (Table 1) namely, SPO11 rs3764674C>T, MN650122 del A, MN708963 ins A, MN708964 ins A, MN720359T>A, RNF212 MN737491delA, rs4045481C>T and SYCP3 rs10860779T>G, MN901901delA and MN995822delA (Fig. 1). Genotype frequency and odd ratios are represented in Table 1.

Among 708 cases, 501 men carried at least one of these variations, showed increased odds in favour of cases over controls. In contrast, only 74 out of 640 controls exhibited any of these variants (excluding rare novel mutations), with no significant association (Table 1). Notably, 12 individuals displayed co-occurrence of at least six variants from the three genes, a rare and novel observation (Table 2) and these individuals, despite receiving hormone therapy (FSH and hCG-based protocols over a 3-month period), were clinically unresponsive, which indicates a likely irreversible spermatogenic failure due to underlying multiple genetic defects. All the genotypes exhibited Hardy-Weinberg equilibrium. Fisher’s exact test revealed a higher prevalence of

polymorphic variants and mutations among azoospermic cases, and we designated these as “risk alleles.”

Each vertical series represents the genotype of a given azoospermic men in respect to ten detectable genetic variants. The ‘+’ signifies presence of genetic variants and ‘-’ signifies absence or wild type condition of the given variants.

The SPO11 intronic variant rs3764674 T>G (ClinGen CA636199486) showed elevated odds in favour of azoospermic cases (odds ratio 30.708, 95% CI 21.131–44.625, RR 9.809, $P<0.0001$). These variant overlaps regulatory regions ENSR00000299043 (CTCF binding site) and ENSR00001053148 (TF binding site). The RNF212 stop-gain variant rs4045481 C>T (ClinGen CA2803542, COSMIC ID COSV61363712) was found in 74 cases (11%) but not in controls. It creates a premature stop codon at amino acid 58, truncating the RNF212 protein and altering splice sites due to its location in exon 3. The SYCP3 intronic variant rs10860779 (ClinGen CA13630093, COSMIC ID COSV57149213) exhibited an elevated odds in favour of azoospermia. (odds ratio 33.081, 95% CI 23.067–47.441, RR 9.739, $P<0.0001$). The RNF212 MN720359 T>A intronic variant and significantly increases the odds in favor of azoospermic cases by ~ 29 folds. (OR=28.489; 95% C.I- 19.072–42.555, RR-10.072, 95% C.I of RR- 7.065–14.360, P value <0.0001). SPO11 MN650122delA, MN708963insA, MN708964insA, MN737491delA, SYCP3 MN901901delA, and MN995822delA variations were discovered to be extremely rare in case samples (frequency among case samples are 3%, 4%, 2.5%, 4%, 5%, and 6% respectively) and completely absent in controls.

Analysis of gene X environmental factors as risk of azoospermia

We stratified ‘case’ and ‘control’ subjects by genetic background: wild type (WT, no variants/mutations in the three genes) and variant carriers (MUT, detected variants) (Table 3). Further, we subcategorized them by age at infertility reporting to laboratory (‘young’ and ‘old,’ as defined in the Methods). Eight interaction models combining age, radiation exposure, and genetic makeup were designed, using ‘young X radiation non-exposed X WT’ as the reference model (Table 3). We found significant elevated odds in favor of azoospermia in four different models: young x radiation non-exposed x MUT (OR 7.71; $P<0.001$), old x radiation non-exposed x MUT (OR 2.6; $P=0.002$), young x radiation exposed x MUT (OR 26.32; $P<0.001$), old x radiation exposed x MUT (OR 24.98; $P<0.001$) (Table 3). Figure 2 represents the binary logistic regression model illustrating the predicted probability of infertility with increasing age across different experimental groups.

Table 1 Association of SPO11, RNF212 and SYCP3 risk variants with the azoospermic ($N=708$) and control samples ($N=640$)

VARIANTS	Gene	TYPE	LOCATION	AMINO ACID CHANGE	BASE POSITION	GENOTYPES	CONTROL (frequency)	CASE (frequency)	ODDS RATIO	95% C.I.†	P VALUE	Relative risk	C.I. of RR†
							$N=640$	$N=708$					
rs3764674 T>G	SPO11	Intron variant	Intron 7–8	N.A	20:57335486	TT	605(0.94)	255 (0.36)	Reference	-	-	-	-
						TG	35 (0.06)	453 (0.64)	30.708	21.131–44.625	<0.0001*	9.809	7.107–13.538
MN650122delA	SPO11	Splice junction/Intron/NMD variant	Intron 6–7	N.A	20:57335414	GG	0 (0.00)	0 (0.00)	-	-	-	-	-
						AA	640 (1.00)	687 (0.97)	Reference	-	-	-	-
MN708963insA	SPO11	Intron variant	3' UTR	N.A	20:57343841	A ₋	0 (0.00)	21 (0.03)	-	-	-	-	-
						AA	0 (0.00)	0 (0.00)	-	-	-	-	-
						A ₋	640 (1.00)	682 (0.96)	Reference	-	-	-	-
						AA	0 (0.00)	26 (0.04)	-	-	-	-	-
MN708964insA	SPO11	Intron variant	3' UTR	N.A	20:57343773	AA	0 (0.00)	0 (0.00)	-	-	-	-	-
						A ₋	640 (1.00)	691 (0.975)	Reference	-	-	-	-
						AA	0 (0.00)	17 (0.025)	-	-	-	-	-
MN720359 T>A	SPO11	Intron variant	3' UTR	N.A	20:57343532	TT	611 (0.95)	301 (0.42)	Reference	-	-	-	-
						TA	29 (0.05)	407 (0.58)	28.489	19.072–42.555	<0.0001*	10.072	7.065–14.360
rs405481 C>T	RNF212	Stop gained	Exon 3	T58*	4:1096837	AA	0 (0.00)	0 (0.00)	-	-	-	-	-
						CC	640 (1.00)	634 (0.89)	Reference	-	-	-	-
						CT	0 (0.00)	74 (0.11)	-	-	-	-	-
						TT	0 (0.00)	0 (0.00)	-	-	-	-	-
MN737491 delA	RNF212	Intron variant	Intron 2–3	N.A	4:1096901	AA	640 (1.00)	684 (0.96)	Reference	-	-	-	-
						A ₋	0 (0.00)	24 (0.04)	-	-	-	-	-
						CC	0 (0.00)	0 (0.00)	-	-	-	-	-
rs10860779 C>A	SYCP3	Intron variant	Intron 6–7	N.A	12:101733484	CA	601 (0.93)	225 (0.31)	Reference	-	-	-	-
						AA	39 (0.07)	483 (0.69)	33.081	23.067–47.441	<0.0001*	9.739	7.180–13.210
MN901901 delA	SYCP3	Intron variant	Intron 6–7	N.A	12:101733828	A ₋	0 (0.00)	0 (0.00)	-	-	-	-	-
						AA	640 (1.00)	676 (0.95)	Reference	-	-	-	-
						AA	0 (0.00)	32 (0.05)	-	-	-	-	-
						AA	0 (0.00)	0 (0.00)	-	-	-	-	-
MN995822 delA	SYCP3	Intron variant	Intron 6–7	N.A	12:101731878	A ₋	640 (1.00)	667 (0.94)	Reference	-	-	-	-
						AA	0 (0.00)	41 (0.06)	-	-	-	-	-
						AA	0 (0.00)	0 (0.00)	-	-	-	-	-

† C.I, Confidence Interval. *Significance level is <0.05 for P value. Power Calculation was done for Fisher's exact test using G*power software

Fig. 1 Chromatograms of MN650122delA, MN708963insA, MN708964insA, MN720359T>A, MN737491delA, MN901901delA, MN995822delA, rs4045481C>T, rs3764674T>G and rs10860779G>T genetic variants

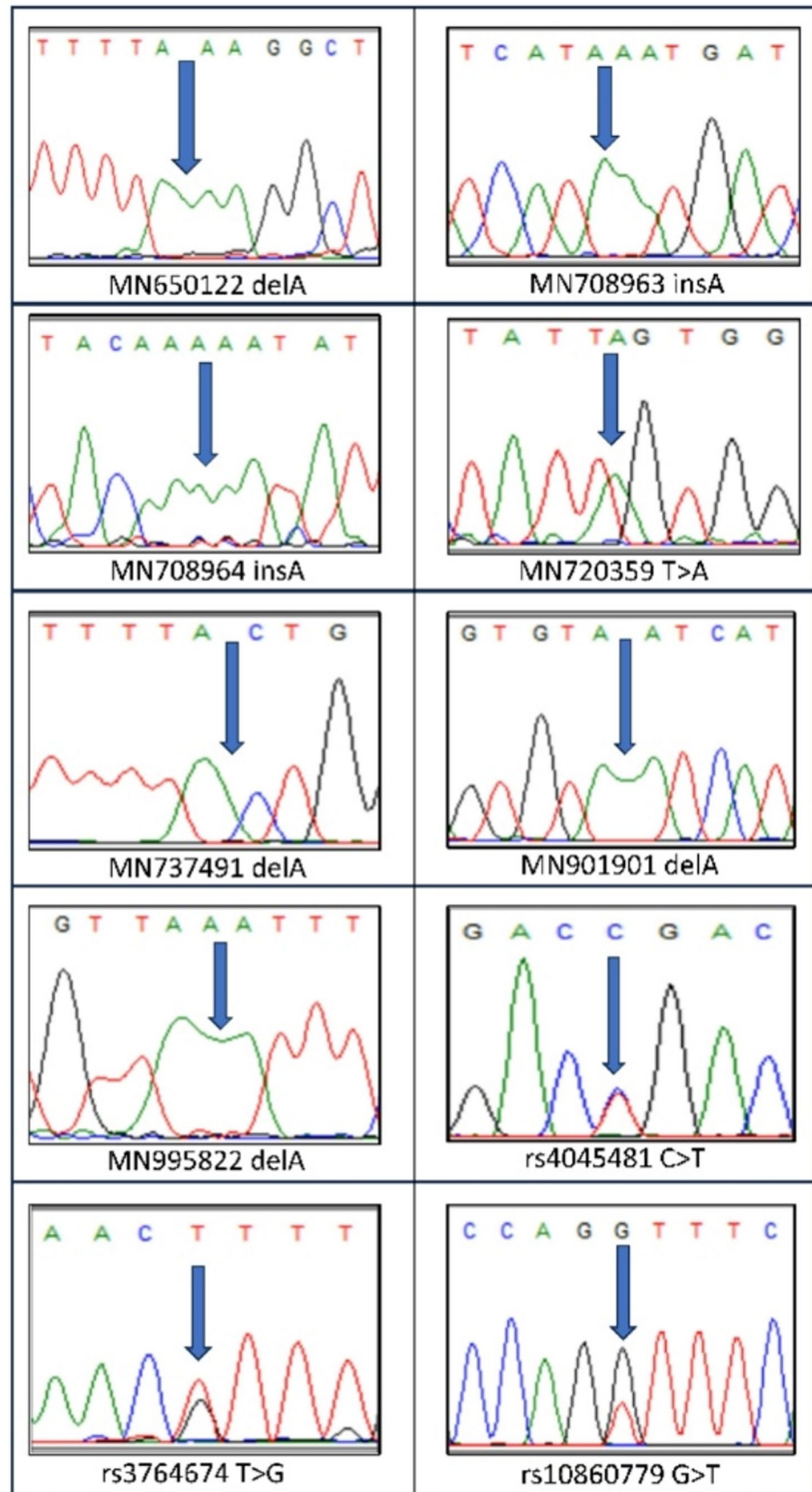


Table 2 Co-occurrence of multiple genetic variants of the genes SPO11, RNF212, SYCP3 among 12 azoospermic individuals

Meiotic Regulator Genes	Genetic variants	Clinical samples											
		MIF 20	MIF 21	MIF 53	MIF 62	MIF 68	MIF 72	MIF 73	MIF 83	MIF 86	MIF 87	MIF 88	MIF 90
SPO11	rs3764674 T>G	+	+	+	+	+	+	+	-	+	+	+	+
	MN650122delA	+	+	+	+	+	-	+	+	+	+	+	+
	MN708963insA	+	+	+	+	-	-	+	+	-	-	-	+
	MN708964insA	+	+	+	+	+	+	-	+	+	+	+	+
	MN720359 T>A	+	+	+	+	+	+	+	+	+	+	+	-
RNF212	rs4045481 C>T	+	+	+	+	+	+	+	+	+	-	+	+
	MN737491 delA	+	+	+	+	+	+	+	+	+	+	+	+
SYCP3	rs10860779 C>A	+	-	+	+	+	-	+	+	+	+	+	+
	MN901901 delA	+	+	+	+	+	+	+	-	-	+	+	+
	MN995822 delA	+	-	+	+	+	+	-	+	+	+	+	+
Phenotype		AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ	AZ

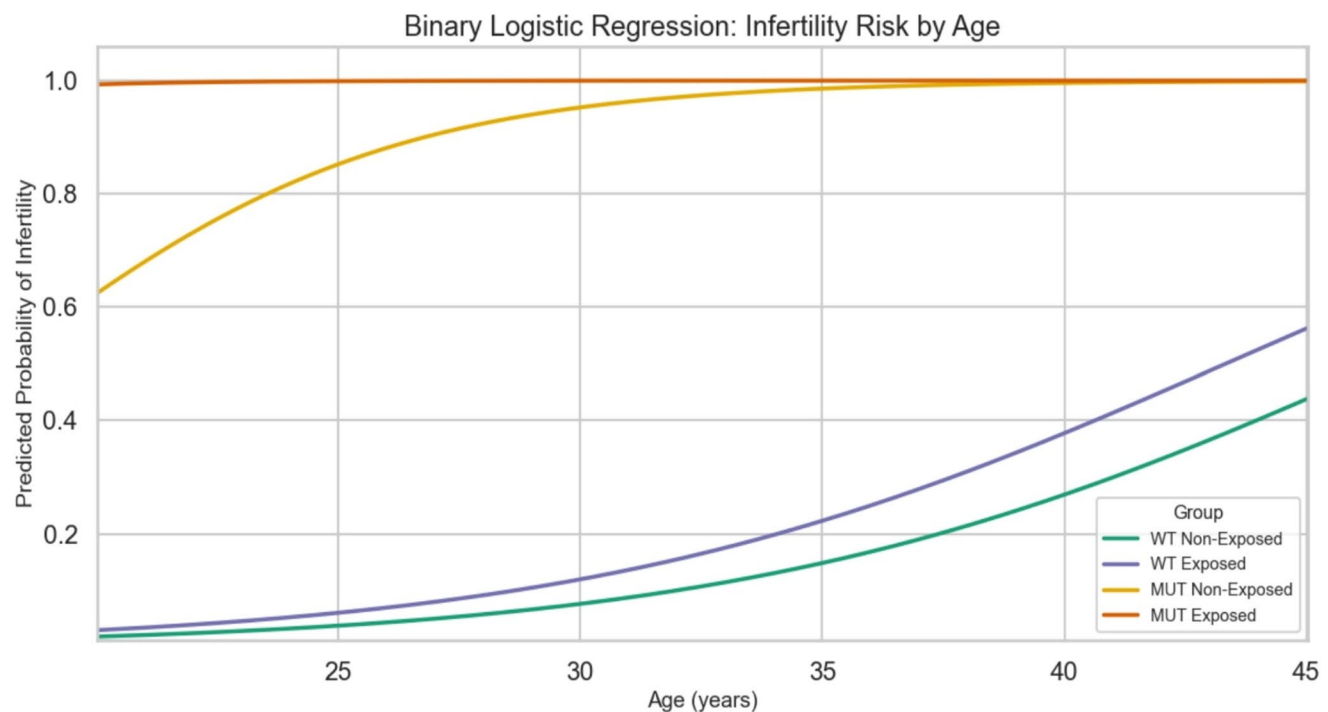
Binary logistic regression analysis was performed considering individual's age, exposure to radiation status and Meiotic regulator mutant genotype as predictor variables and azoospermia as outcome variable where P value < 0.05 is considered statistically significant. N=number of individuals, OR=odd ratio, CI=confidence interval, Meiotic Regulator -WT=Meiotic regulator wild-type genotype, Meiotic Regulator-MUT=Meiotic regulator polymorphic/mutant genotype.

Discussion

The etiology of male infertility is multifactorial. Besides, known genetic challenges, lifestyle risks (also known as environmental or habitual risk factors) have already been recognized as risk factors for male infertility [18, 19, 21–23, 37]. This study provides strong evidence that specific genetic variants in core meiotic regulators, particularly when accompanied by environmental exposure to non-ionizing radiation, significantly increase the risk of azoospermia. The findings are consistent with existing literature on the role of SPO11, RNF212, and SYCP3 in gametogenesis and support the emerging view that gene-environment interplay is central to complex reproductive disorders. A handful of researches have reported the negative impact of lifestyle hazards on both male and female reproductive outcome [28, 31, 38–41]. Our group has previously characterized that AZFc partial deletions combined with smokeless chewing tobacco use, increase the risk of infertility in men [42]. For the first time, we designed a reliable 'G x E' model to explore the etiology of male infertility, utilizing the considerable large sample cohort in an epidemiology study and unique data on radiation exposure from electronic devices. We identified novel mutations and polymorphic variants in SPO11, RNF212, and SYCP3 associated with infertility in Bengali-speaking azoospermic men. Novel variants (SPO11 MN650122delA, MN708963insA, MN708964insA, MN720359T>A, RNF212 MN737491delA, and SYCP3 MN901901delA, MN995822delA) and previously reported ones (SPO11 rs3764674T>G, RNF212 rs4045481C>T, SYCP3 rs10860779C>A) were significantly prevalent among cases. Additionally, we have identified 12 subjects who carried multiple genetic variants/mutations. This novel observation suggests possible synergistic effects among variants, potentially disrupting DNA repair. These individuals were detected unresponsive to any kind of clinical interventions, represent extreme cases of azoospermia. It is still a mystery how these non-coding genetic variations can increase an individual's risk of disease manifestation. Almost all of the genetic variations that we identified are not located in protein-coding areas. Recent scientific findings

Table 3 Interactions among the variants of SPO11, RNF212, SYCP3, age and radiation exposure that increase risk of azoospermia

Case	Genotype	Exposure to Radiation	Age	Frequency (%)	Regression analysis			
					Interactions (maternal age × SCT use status × polymorphism status)	OR	95% CI	P value
(N=708)	Meiotic Regulator -WT (N=207)	Non-Exposed (N=118)	Young age group (≤30 yrs.)	0.27	Young age group × Radiation non-exposed × meiotic regulator-WT	Reference		
			Old age group (≥30 yrs.)	0.30	Old age group × Radiation non-exposed × meiotic regulator-WT	0.775	0.50–1.19	0.249
		Exposed (N=89)	Young age group (≤30 yrs.)	0.16	Young age group × Radiation-exposed × meiotic regulator-WT	0.630	0.37–1.04	0.075
			Old age group (≥30 yrs.)	0.27	Old age group × Radiation-exposed × meiotic regulator-WT	0.676	0.43–1.04	0.080
	Meiotic Regulator -MUT (N=501)	Non-Exposed (N=82)	Young age group (≤30 yrs.)	0.10	Young age group × Radiation non-exposed × meiotic regulator-MUT	7.714	3.86–15.38	<0.001
			Old age group (≥30 yrs.)	0.07	Old age group × Radiation non-exposed × meiotic regulator-MUT	2.630	1.44–4.78	0.002
		Exposed (N=419)	Young age group (≤30 yrs.)	0.25	Young age group × Radiation-exposed × meiotic regulator-MUT	26.324	12.83–53.99	<0.001
			Old age group (≥30 yrs.)	0.58	Old age group × Radiation-exposed × meiotic regulator-MUT	24.984	14.79–42.20	<0.001

**Fig. 2** Binary logistic regression plot showing the predicted probability of infertility across age groups (20–45 years) for four different cohorts: WT Non-Exposed, WT Exposed, MUT Non-Exposed, and MUT Exposed. Infertility risk increases with age in all groups, with the highest probability observed in the MUT Exposed group (approaching

1.0 across all ages), followed by MUT Non-Exposed. WT groups show a comparatively lower risk, with exposure contributing to a moderate increase in infertility probability. This trend highlights the synergistic effect of mutation and exposure on age-related infertility risk

have shed light on the significance of non-coding genetic variations in the development of a variety of disorders. Contrary to their earlier label as ‘junk DNA’, these non-coding sequences are now recognized as important players in gene regulation and disease vulnerability [40, 41].

It is important to note that the majority of azoospermic individuals in our cohort were clinically classified as

non-obstructive azoospermia based on semen volume, hormonal profiling, and absence of palpable vasal or epididymal obstruction. Therefore, the role of radiation exposure in obstructive pathology was not directly assessed. While speculative, any potential association between radiation and obstructive defects would likely require evidence of fibrosis

or inflammatory responses in the reproductive tract, which was beyond the scope of this study.

Using gene–environment ($G \times E$) interaction models, we evaluated the combined effects of age and radiation exposure from electronic devices with genetic variants (including both polymorphisms and mutations) of key meiotic regulator genes to identify how putative risk alleles associate epidemiological factors with male infertility. We observed a significant increase in risk of infertility in young and older age group when both gene variants and radiation exposure were present. Gene variants alone elevated azoospermia risk (odds ratio: 7.71 in younger, 2.63 in older), which was further exacerbated many folds with radiation exposure (odds ratio: 26.23 in younger, 24.98 in older; Table 3).

Future studies are needed to confirm these findings and to explore underlying mechanistic detail. Effect of electromagnetic field generated by mobile phone or laptop on sperm production, and man's sexual health have been studied in preclinical mice models [42, 43] and on clinical samples [44–46]. Our stratified regression analysis revealed that the risk of azoospermia was significantly increased in subjects carrying meiotic regulator variants, with the effect being substantially intensified among those exposed to radiation. This trend was consistent across both age groups, supporting a robust **GxE interaction** contributing to infertility. The outcome of all those studies revealed impairment in sperm production under the exposure electromagnetic field of mobile phone or laptop or both. Practice of keeping mobile phone on trouser pocket may create a high potential electromagnetic field and elevated temperature around the male genitalia including testicle which progressively increases the risk of infertility. Adverse effect of laptop or cell phone uses causing fertility and erectile dysfunction were reported previously [45].

Our study has some limitations. First, we may have missed other genetic variants in meiotic regulator genes beyond the identified ten variants that we analyzed. Next-generation sequencing (NGS) could help to identify additional deeply seated genetic variants. Second, we focused on a single environmental risk factor (radiation from electronic devices) to simplify the analysis, though many other environmental challenges may act synergistically. Third, we did not account for clinical information such as hormone profiles and metabolic conditions in statistical analyses, which could further shed light on the concept of multifactorial effects on meiosis dysregulation. Also, testicular biopsies or TESE samples could not be obtained because of ethical, clinical, and procedural limitations in a non-therapeutic, observational study design. Despite being educational, these invasive procedures are usually only recommended during ART or surgical sperm retrieval protocols. We were ethically restricted to non-invasive sampling because our study was

focused on genetic epidemiology and included only willing participants who were not undergoing surgical fertility treatment. We recognize that testicular tissue analysis, either by gene expression profiling or histopathology, would greatly improve our comprehension of how the detected variants impair spermatogenesis. Testis biopsies will be essential for mechanistic correlation and functional validation in future research, especially in MRV+ individuals.

Conclusion

In conclusion, our findings demonstrate that men harboring specific genetic variants in key meiotic regulator genes face significantly elevated risk of azoospermia, particularly when exposed to non-ionizing electronic radiation. This study underscores the importance of integrating genetic screening and lifestyle evaluation in infertility diagnosis and ART planning. We acknowledge the critical need to study the expression and regulatory effects of these variants within gonadal tissue, where the direct impact on spermatogenesis could be observed. Evaluating these variants in testicular biopsy specimens or germ cells, especially under common environmental exposures such as electronic radiation, could reveal functional consequences such as aberrant gene expression, splicing defects, or histological anomalies. Such tissue-specific analyses are warranted in future studies to clarify the mechanistic link between the detected variants and spermatogenic failure.

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Author contributions Sujay Ghosh and Samudra Pal conceptualized the study and supervised the overall project. Samudra Pal performed the experiments, curated the data, and carried out the formal analyses with contributions from Papiya Ghosh, Saurav Dutta, and Pranab Paladhi. The methodology was developed and refined by Samudra Pal, Saurav Dutta, Pranab Paladhi, Papiya Ghosh, and Ratna Chattopadhyay. Samudra Pal and Sujay Ghosh developed and implemented the software tools used in the study. Project administration, validation, and funding acquisition were overseen by Sujay Ghosh. Samudra Pal prepared the original draft of the manuscript, with contributions from Sujay Ghosh and Papiya Ghosh. The manuscript was critically reviewed and edited by Sujay Ghosh.

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Data availability The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval and consent to participate The present study protocol was reviewed and approved by the institutional review board of University of Calcutta, West Bengal, Kolkata, India (Dated 04/12/2017; Approval No. CU/BIOETHICS/HUMAN/2306/3044). We followed the criteria outlined in the “Declaration of Helsinki” and the Indian Council of Medical Research (ICMR). Informed written consent was submitted by all subjects when they were enrolled.

Animal research Not Applicable.

Clinical trials Not Applicable.

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