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Original Article

Whole-genome sequencing identifies a potentially causative homozygous splicing variant in *RAD54* like in non-obstructive azoospermia

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Abstract

Background: Non-obstructive azoospermia (NOA) is a severe form of male infertility characterized by impaired spermatogenesis. Although chromosomal abnormalities and Y-chromosome microdeletions are known causes, many cases remain idiopathic, suggesting that other genetic factors may be involved. RAD54 like (*RAD54L*) is a key component of the homologous recombination pathway required for DNA double-strand break repair and meiotic progression.

Objective: To identify the potential genetic cause of NOA using whole-genome sequencing (WGS) in affected individuals from consanguineous families.

Materials and Methods: For performing this experimental study, 10 NOA men who were referred to Infertility Center of the Yazd Reproductive Sciences Institute, Yazd, Iran underwent clinical, hormonal, and genetic evaluations. Genomic DNA was extracted from peripheral blood and was subjected to WGS. Variants were filtered and prioritized based on allele frequency, predicted pathogenicity, inheritance pattern, and gene expression data. In silico splicing analyses were performed using SpliceAI and Human Splicing Finder.

Results: WGS identified an ultrarare homozygous splice-site variant, c.1375+1G>T, in the *RAD54L* gene. The variant affects a canonical donor splice site and is predicted to cause loss of normal splicing, likely resulting in exon 12 skipping, frameshift, and premature termination codon formation. *RAD54L* shows the highest expression in testicular tissue and plays a critical role in homologous recombination and meiotic DNA repair. The patient exhibited hormonal findings consistent with primary testicular failure.

Conclusion: This study proposes *RAD54L* as a potential novel candidate gene linked to NOA and underscores the efficacy of WGS in identifying rare genetic etiologies of idiopathic male infertility. Further functional and clinical studies are needed to validate this association.

Keywords: *Infertility, Azoospermia, RAD54L, Spermatogenesis, Whole genome sequencing, Splice-site variant.*

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1. Introduction

Infertility is the inability to conceive after 1 yr of regular unprotected intercourse. It affects about 186 million people worldwide (1). Male infertility accounts for nearly 50% of infertility cases (2). Non-obstructive azoospermia (NOA), which is characterized by the absence of sperm in ejaculate despite no obstruction, affects about 1% of men and represents 15% of infertility cases (3, 4). More than 70% of NOA cases are idiopathic. Most NOA cases have primary testicular failure. The 3 most common histological classifications of NOA are Sertoli cell-only syndrome, hypospermatogenesis, and maturation arrest (5).

While chromosomal abnormalities and Y-chromosome microdeletions are well-established genetic causes, a significant proportion of NOA cases remain idiopathic, indicating a complex genetic heterogeneity. Many cases of NOA still lack a clear genetic explanation, highlighting the need for continued research into novel genes and regulatory mechanisms (6). A percentage of NOA cases are caused by monogenic mutations. More than 30 potential genes linked to NOA have been found using whole-exome sequencing (7). Whole-genome sequencing (WGS) provides a more comprehensive approach by detecting variations in both coding and non-coding regions, such as single nucleotide variants, insertions/deletions, and structural variants (8).

Spermatogenesis involves approximately 4000 genes. However, monogenic causes of NOA remain poorly understood. This is largely due to the high genetic heterogeneity associated with the complex processes of spermatogenesis and testicular function, along with the limited availability of analysis of consanguineous familial cases and validation studies (9). Consanguineous families could be especially appropriate for genetic research aimed at identifying genetic variants. Offspring of consanguineous marriages are more likely to inherit 2 copies of a rare deleterious allele, which can result in homozygous recessive disorders (10).

Future research will continue to leverage whole-exome sequencing and WGS to uncover more monogenic causes, potentially identifying new biomarkers for diagnostic and prognostic purposes (11, 12). In this study, we used WGS to identify potential genetic etiology of NOA in 10 males.

2. Materials and Methods

2.1. Patient selection and sample preparation

For this experimental study in 2023, 10 men from consanguineous families were referred to the Infertility Center of the Yazd Reproductive Sciences Institute, Yazd, Iran. They had normal karyotypes, no Y-chromosome microdeletions, and no history of drug abuse, varicocele, testicular torsion, orchitis, or cryptorchidism. A 10 ml whole-blood sample was collected in sterile, single-use ethylenediaminetetraacetic acid-containing tubes for genomic DNA extraction from each participant. DNA was isolated using the High Pure Polymerase Chain Reaction Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the manufacturer's instructions. DNA concentration and purity were evaluated using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham,

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MA, USA) and a Quantus fluorometer (Promega, Madison, WI, USA). The purified DNA samples were stored at -20°C until further analysis.

2.2. WGS and variant prioritization

The genomic DNA samples were sequenced on the Illumina NovaSeq X platform, with an average depth of ×30 (~120 Gbp). Sequencing quality was checked with FastQC (v0.12.0). Raw FASTQ files were processed using cgpmap. Reads were aligned to the GRCh38 reference genome using Burrows-Wheeler Aligner-Maximal Exact Matches. Duplicate reads were marked with biobambam2, and post-processing was performed using SAMtools (13–15). Variants were called using DeepVariant (v1.6.0), and only variants that passed the internal quality filters were retained (16). VCFtools (v0.1.16) was used to filter variants by applying a minimum quality score ≥ 30, a mean read depth (DP) ≥ 10×, and a genotype call rate of ≥ 90% (17).

Variants were annotated using Ensembl Variant Effect Predictor (release 112) (18). Pathogenicity scores from dbNSFP (version 4.4a) were added using SnpSift (v5.2) (19, 20). Variants with a genome allele frequency of 1% or higher in gnomAD were excluded. Variants without gnomAD frequency data were kept to detect potentially novel rare variants. Further analyses focused on variant type, inheritance pattern, and pathogenicity predictions from MutationTaster. Gene expression data were evaluated using the genotype-tissue expression (v7) database.

We reviewed the literature using PubMed to see how candidate genes might affect fertility. Gene IDs combined with keywords such as "spermatogenesis", "infertility", "azoospermia", and "reproductive biology" were searched. Additionally, copy-number variants were detected using ClinSV (v1.0) and assessed for a comprehensive analysis of genomic alterations associated with male infertility (20).

2.3. Splicing prediction

To evaluate the potential impact of the variant on canonical 5′ donor and 3′ acceptor splice sites, as well as to predict the creation or activation of cryptic splice sites, in silico analyses were performed using SpliceAI, a deep learning-based splicing prediction tool, and human splicing finder (HSF) (21, 22).

2.4. Sanger sequencing

To validate the candidate variants identified through WGS, Sanger sequencing was performed using an ABI 3500XL genetic analyzer. Analyses were performed with Finch TV software (v1.4.0). The following *RAD54L* primer sequences (5′-3′) were used: forward, [F: TCCTGTTTTCTCTAGATGCCT]; reverse, [R: TGCCCAACATTGTATATGAGC].

2.5. Ethical Considerations

Written informed consent was obtained from the NOA cases for genome sequencing and other described studies. Sample collection was conducted under protocols approved by the working group of the ethics committee in research of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Code: IR.SSU.MEDICINE.REC.1402.124).

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3. Result

3.1. Identified potential causative variant

We performed WGS to find potential causative single nucleotide variants or copy number variants. We identified an ultrarare homozygous splicing variant c.1375+1G>T, in the RAD54 like (*RAD54L*) gene, in one of the analyzed cases. This variant lies within the consensus donor splice sequence of intron 12 (Table I). The BAM file and Sanger sequencing result confirm homozygosity of this variant (Figures 1A and 1B). Among human tissues, *RAD54L* displays the highest expression level in the testis, with 17.35 transcripts per million value (https://gtexportal.org/home/gene/RAD54L).

3.2. Splicing prediction

In silico splicing analysis using SpliceAI (donor loss score = 0.96) and HSF predicted a high-confidence loss of the canonical donor splice site downstream of exon 12, with no evidence for compensatory cryptic splice site activation. The most likely consequence is exon 12 skipping (131 bp), causing a frameshift and premature termination codon, potentially leading to nonsense-mediated decay, or yielding a truncated protein (Figure 2).

3.3. Clinical characteristics

This splicing variant (c.1375+1G>T) in *RAD54L* was detected in a 36-yr-old man with NOA. He originated from a consanguineous family with first-cousin parents (Figure 3). Semen analysis was consistent with NOA (Table II). His physical examination and karyotype (46, XY) were normal with no Y-chromosomal microdeletions. Hormonal evaluation revealed a significant elevation of serum follicle-stimulating hormone (FSH) and a mild increase in luteinizing hormone. The testosterone level was within the normal reference range but close to its lower limit. Thyroid-stimulating hormone and prolactin concentrations were within the normal limits (Table III).

Table I. Information of the identified potential disease-causing variant

Gene	Chromosomal position (GRCh38)	mRNA/cDNA change/protein change	In silico prediction scores (CADD/mutation taster)	ACMG classification	Allele frequency (gnomAD v4/) number of homozygotes
RAD54L	chr1 46272803 G T	(NM_003579.4):c.1375+1G>T p.?	Pathogenic (33)/Disease causing	Likely pathogenic (PVS1+PM2)	0.00000657 /0

GRCh38: Genome reference consortium human build 38, mRNA: Messenger ribonucleic acid, cDNA: Complementary deoxyribonucleic acid, CADD: Combined annotation dependent depletion, ACMG: American college of medical genetics and genomics, PVS1: Pathogenic very strong evidence 1, PM2: Pathogenic moderate evidence 2, gnomAD: Genome aggregation database

Table II. Semen analysis parameters of the male with non-obstructive azoospermia

Test	Results	Unit	Reference range
Abstinence duration	3	Day	2–7
Liquefaction time	15	Minute	Less than 60 min

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Table II. Continued

Test	Results	Unit	Reference range
Volume	5.2	ml	> 1.5
Appearance	clear	-	-
Color	Opalescent	-	Grey-opalescent
PH	7.2	-	7.2–8.0
Agglutination	None	-	None
Viscosity	Low	-	-
Sperm concentration	Zero	Mil/ml	> 15

pH: Potential of hydrogen

Table III. Endocrine parameters of the male with non-obstructive azoospermia

Studied hormones	Results	Unit	Reference range
FSH	42.3	mIU/ml	1.5–13
LH	12	mIU/ml	1.2–9.0
Testosterone	3.4	ng/ml	2.8–8.0
TSH	3.8	µIU/ml	0.3–4.6
Prolactin	8.23	ng/ml	2.5–17

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone

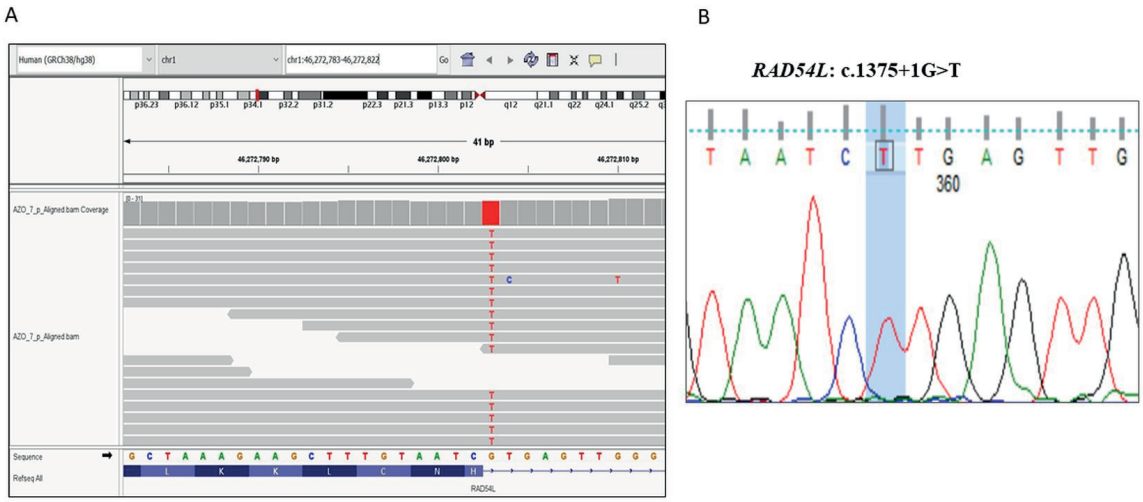

Figure 1. Integrative genomics viewer (IGV) snapshot showing the BAM file of the *RAD54L* c.1375+1G>T splice-site variant and corresponding Sanger sequencing validation. A) The BAM file demonstrates a G>T substitution at the canonical +1 donor splice site (c.1375+1G>T) of the *RAD54L* gene. All reads show the alternate allele (T), with no evidence of the reference nucleotide, consistent with a homozygous variant. B) The Sanger sequencing chromatogram validates the homozygous (T/T) genotype of *RAD54L* candidate c.1375+1G>T variant.

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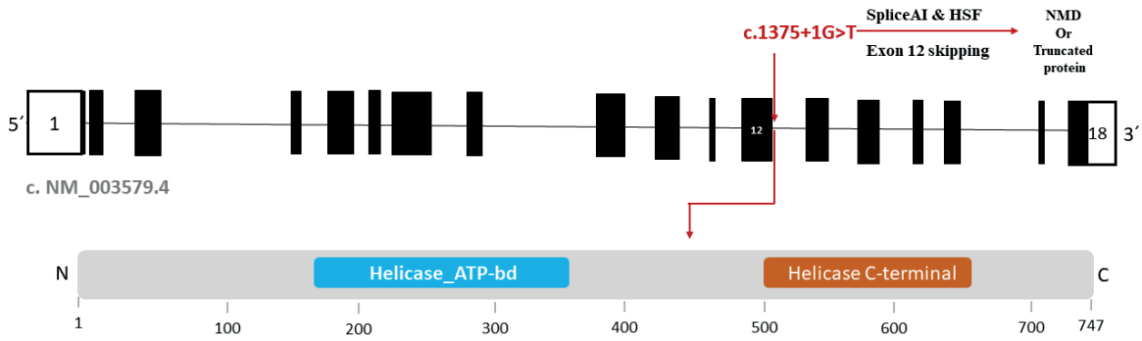

Figure 2. Schematic representation of the *RAD54L* gene structure, localization of the c.1375+1G>T variant, and its predicted functional consequences. The variant is located at the canonical donor splice site after exon 12 of transcript NM_003579.4. In silico tools (SpliceAI and HSF) predict exon 12 skipping, potentially resulting in a frameshift, premature stop codon, and subsequent nonsense-mediated decay (NMD) or truncated protein production. The lower panel shows the Helicase ATP-binding and Helicase C-terminal domains of the RAD54L protein.

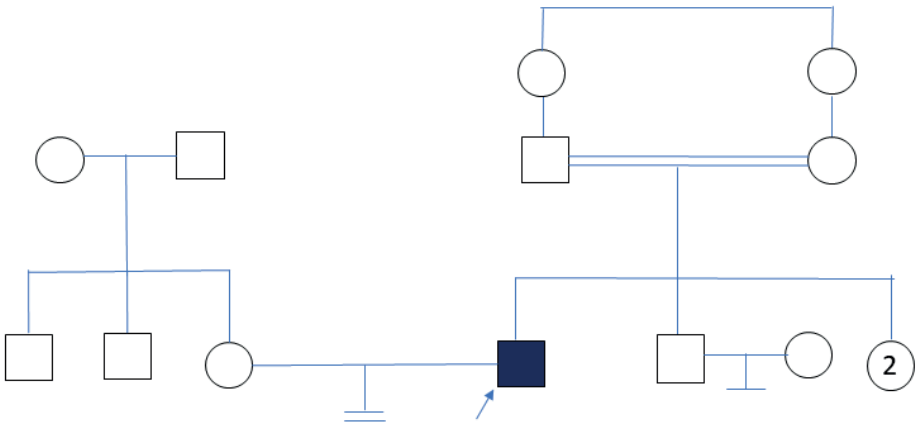

Figure 3. Pedigree of the man with NOA showing consanguineous family. The proband was born to first-cousin parents, indicated by a double horizontal line, consistent with a consanguineous marriage. His brother has no offspring, with no available information regarding the underlying etiology.

4. Discussion

In this study, we found a homozygous splicing variant (c.1375+1G>T) in *RAD54L* in a man diagnosed with NOA from a consanguineous family. Splicing prediction tools indicated that this substitution disrupts normal splicing at the exon 12-intron boundary. Due to the absence of a strong alternative donor site, exon 12 skipping is the most likely outcome, which probably results in a frameshift and introduces a premature termination codon.

RAD54L is a crucial DNA motor protein involved in the homologous recombination (HR) pathway of DNA double-strand break (DSB) repair. The ATPase RAD54L is required for the deposition and stabilization of RAD51 foci at DSBs. It also contributes to chromatin remodeling during HR and promotes dissociation of RAD51 (23, 24). This process is fundamental for maintaining genomic integrity and successful meiosis (25). RAD54L and RAD54B both interact with RAD51 in mammalian cells but have non-overlapping functions. RAD54L is required for proper RAD51 recruitment to meiotic DSBs, whereas RAD54B primarily interacts with DMC1. Therefore, despite their homology, these proteins are not functionally interchangeable (26–29).

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RAD54L is essential for normal germ cell development in both male and female mice. Its deficiency leads to impaired primordial germ cell development and increased apoptosis. Although partial germ cell depletion does not markedly affect fertility, *RAD54l*-deficient males exposed to prenatal genotoxic stress develop complete sterility. These findings highlight the critical role of *RAD54L* in preserving premeiotic germ cell viability following DNA damage, while Sertoli cell survival appears to be *RAD54L*-independent (30).

Dysregulation of DNA repair mechanisms, including HR, is increasingly linked to male infertility in both mouse models and humans (30, 31). RAD51 mediates HR repair in mitosis and meiosis. A conditional knockout mouse model showed that *RAD51* was highly expressed in germ cells of the mouse testis. Conditional disruption of *RAD51* in germ cells led to a Sertoli cell-only phenotype (32).

RAD54L works downstream of the recombinase RAD51 in HR and is known to regulate replication fork progression and nascent strand degradation, especially in cells deficient in BRCA1/2, another HR pathway component (25). Given that HR is essential for successful meiotic recombination and accurate chromosome segregation during spermatogenesis (31, 33), any deficiency in RAD54L would likely lead to meiotic arrest and spermatogenic failure, mirroring findings for other *HR* genes. For instance, bi-allelic variants in *SHOC1*, a gene that plays a key role in HR and crossing over in meiotic prophase I in male and female germ cells, cause NOA with meiotic arrest in both humans and mice (31). Similarly, mutations in genes like *FANCM* and *TEX11*, also involved in DNA repair and meiotic processes, have been linked to Sertoli cell-only syndrome and NOA in humans (34, 35).

Research indicates that *RAD54L* is frequently overexpressed in various cancer types, and this overexpression is often associated with poorer prognosis. For instance, high *RAD54L* expression has been linked to bladder cancer progression, affecting cell cycle regulation and senescence (36). Similarly, in hepatocellular carcinoma, elevated *RAD54L* expression promotes disease progression, and has been identified as a prognostic biomarker (37). Some studies have identified somatic and germline mutations in one allele of *RAD54L* in non-Hodgkin's lymphoma, breast, and colorectal cancers (38).

In Rad54-/- male mice, mild reproductive effects, including a reduced number of spermatogonia in adult testes and decreased recombination efficiency, have been detected (30). Furthermore, during meiotic prophase I, abnormal RAD51 distribution has been observed. Impaired RAD54L function also leads to a marked increase in chromosomal aberrations, some of which persist through meiosis and may result in the generation of unbalanced gametes (39). Although *RAD54L* has the highest expression in the human testis and has been implicated in HR pathways relevant to male infertility, germline variants associated with NOA in humans have not yet been reported. Cancer-associated phenotypes linked to *RAD54L* are typically related to heterozygous or somatic variants, whereas our case has a germline homozygous splice-site variant presenting with an early-onset reproductive phenotype.

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The hormonal profile is consistent with primary testicular failure. The main limitation of this study was the unavailability of other family members for segregation analysis, the lack of micro-TESE results, and the absence of RNA-based functional validation to confirm exon 12 skipping due to the unavailability of fresh samples for RNA extraction.

5. Conclusion

In summary, we identified an ultrarare homozygous splice-site variant (c.1375+1G>T) in the RAD54L gene in a man with NOA from a consanguineous family. In silico analysis strongly predicted disruption of the canonical donor splice site, most likely resulting in exon skipping, frameshift, and premature protein truncation or nonsense-mediated mRNA decay. Since RAD54L is important for HR and DNA DSB repair during meiosis, a defect in this gene could disrupt spermatogenesis and lead to testicular failure.

This is the first study that suggests a germline RAD54L variant as a cause of human NOA. The result of this study expands the range of genes involved in male infertility. These results further emphasize the importance of WGS in identifying rare genetic causes of idiopathic NOA, particularly in individuals from consanguineous backgrounds. Functional studies and validation in larger patient cohorts are necessary to confirm the pathogenic role of RAD54L variants in male infertility and to clarify their clinical implications.

Data Availability

The findings of this study are available upon reasonable request from the corresponding author.

Author Contributions

R. Ebrahimi Askari: Designed the study, prepared the samples, performed variant filtering and interpretation, and drafted the manuscript. A. Malcher: Coordinated the study, contributed to data interpretation, and assisted in editing and finalizing the manuscript. S. Sadeghzadeh: Assisted with sample preparation and data interpretation. R. Ibrahim: Contributed to variant filtering and data interpretation and assisted in editing the manuscript. A. Srivastava and K. Pastuszak: Conducted the bioinformatics analysis. SM. Kalantar: Contributed to patient selection. S. Vahidi: Recruited the patients and reviewed their medical histories. N. Ghasemi: Coordinated the study, supervised the project, and contributed to patient selection. M. Kurpisz: Coordinated and supervised the project and participated in manuscript editing and finalization. All authors read and approved the final version of the manuscript.

The data presented in this manuscript originate from an international collaborative project involving two research centers. Therefore, we have designated two corresponding authors, one from each center.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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References

[1] Feng J, Wu Q, Liang Y, Liang Y, Bin Q. Epidemiological characteristics of infertility, 1990–2021, and 15-year forecasts: An analysis based on the global burden of disease study 2021. *Reprod Health* 2025; 22: 26.

[2] Agarwal A, Baskaran S, Parekh N, Cho C-L, Henkel R, Vij S, et al. Male infertility. *Lancet* 2021; 397: 319–333.

[3] Fakhro KA, Elbardisi H, Arafa M, Robay A, Rodriguez-Flores JL, Al-Shakaki A, et al. Point-of-care whole-exome sequencing of idiopathic male infertility. *Genet Med* 2018; 20: 1365–1373.

[4] Agarwal A, Majzoub A, Parekh N, Henkel R. A schematic overview of the current status of male infertility practice. *World J Mens Health* 2020; 38: 308–322.

[5] Cioppi F, Rosta V, Krausz C. Genetics of azoospermia. *Int J Mol Sci* 2021; 22: 3264.

[6] Kasak L, Laan M. Monogenic causes of non-obstructive azoospermia: Challenges, established knowledge, limitations and perspectives. *Hum Genet* 2021; 140: 135–154.

[7] Ibrahim R, Malcher A, Kurpisz M. Summarizing the human genes and their variants causative of non-obstructive azoospermia uncovered using whole genome/exome sequencing. *Reprod Biol* 2025; 25: 101048.

[8] UK Biobank Whole-Genome Sequencing Consortium. Whole-genome sequencing of 490,640 UK Biobank participants. *Nature* 2025; 645: 692–701.

[9] Cazin C, Neirijnck Y, Loeuillet C, Wehrli L, Kühne F, Lordey I, et al. Combined use of whole exome sequencing and CRISPR/Cas9 to study the etiology of non-obstructive azoospermia: Demonstration of the dispensable role of the testis-specific genes C1orf185 and CCT6B. *Cells* 2021; 11: 118.

[10] Uddin I, Zafar I, Xu C, Li W, Khan MI, Wu L, et al. Whole-exome sequencing identifies rare recessive variants in azoospermia patients from consanguineous Pakistani families. *Mol Genet Genomics* 2024; 299: 111.

[11] Tang D, Lv M, Gao Y, Cheng H, Li K, Xu C, et al. Novel variants in helicase for meiosis 1 lead to male infertility due to non-obstructive azoospermia. *Reprod Biol Endocrinol* 2021; 19: 129.

[12] Zhong Y, Zhao J, Deng H, Wu Y, Zhu L, Yang M, et al. Integrative bioinformatics analysis to identify novel biomarkers associated with non-obstructive azoospermia. *Front Immunol* 2023; 14: 1088261.

[13] Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv* 2013. (Preprint)

[14] Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. *Bioinformatics* 2009; 25: 2078–2079.

[15] Tischler G, Leonard S. Biobambam: Tools for read pair collation based algorithms on BAM files. *Source Code Biol Med* 2014; 9: 13.

[16] Poplin R, Chang P-C, Alexander D, Schwartz S, Colthurst T, Ku A, et al. A universal SNP and small-indel variant caller using deep neural networks. *Nat Biotechnol* 2018; 36: 983–987.

[17] Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. *Bioinformatics* 2011; 27: 2156–2158.

[18] McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GRS, Thormann A, et al. The ensembl variant effect predictor. *Genome Biol* 2016; 17: 122.

[19] Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms; SnpEff. *Fly (Austin)* 2012; 6: 80–92.

[20] Minoche AE, Lundie B, Peters GB, Ohnesorg T, Pinese M, Thomas DM, et al. ClinSV: Clinical grade structural and copy number variant detection from whole genome sequencing data. *Genome Med* 2021; 13: 32.

[21] Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, Darbandi SF, Knowles D, Li YI, et al. Predicting splicing from primary sequence with deep learning. *Cell* 2019; 176: 535–548. e24.

[22] Desmet F-O, Hamroun D, Lalande M, Collod-Bëroud G, Claustres M, Béroud C. Human splicing finder: An online bioinformatics tool to predict splicing signals. *Nucleic Acids Res* 2009; 37: e67.

[23] Bugreev DV, Hanaoka F, Mazin AV. Rad54 dissociates homologous recombination intermediates by branch migration. *Nat Struct Mol Biol* 2007; 14: 746–753.

[24] Bugreev DV, Mazina OM, Mazin AV. Rad54 protein promotes branch migration of holliday junctions. *Nature* 2006; 442: 590–593.

[25] Uhrig ME, Sharma N, Maxwell P, Gomez J, Selemenakis P, Mazin AV, et al. Disparate requirements for RAD54L in replication fork reversal. *Nucleic Acids Res* 2024; 52: 12390–12404.

[26] Sarai N, Kagawa W, Kinebuchi T, Kagawa A, Tanaka K, Miyagawa K, et al. Stimulation of Dmc1-mediated DNA strand exchange by human Rad54B protein. *Nucleic Acids Res* 2006; 34: 4429–4437.

[27] Sehorn MG, Sigurdsson S, Bussen W, Unger VM, Sung P. Human meiotic recombinase Dmc1 promotes ATP-dependent homologous DNA strand exchange. *Nature* 2004; 429: 433–437.

[28] Tanaka K, Kagawa W, Kinebuchi T, Kurumizaka H, Miyagawa K. Human Rad54B is a double-stranded DNA-dependent ATPase and has biochemical properties different from its structural homolog in yeast, Tid1/Rdh54. *Nucleic Acids Res* 2002; 30: 1346–1353.

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[29] Mazin AV, Mazina OM, Bugreev DV, Rossi MJ. Rad54, the motor of homologous recombination. *DNA Repair (Amst)* 2010; 9: 286–302.

[30] Messiaen S, Le Bras A, Duquenne C, Barroca V, Moison D, Déchamps N, et al. Rad54 is required for the normal development of germ cells and contributes to the maintainance of their genome integrity after genotoxic stress. *Cell Death Dis* 2013; 4: e774.

[31] Wang W, Meng L, He J, Su L, Li Y, Tan C, et al. Bi-allelic variants in SHOC1 cause non-obstructive azoospermia with meiosis arrest in humans and mice. *Mol Hum Reprod* 2022; 28: gaac035.

[32] Qin J, Huang T, Wang J, Xu L, Dang Q, Xu X, et al. RAD51 is essential for spermatogenesis and male fertility in mice. *Cell Death Discov* 2022; 8: 118.

[33] Huang T, Wu X, Wang S, Bao Z, Wan Y, Wang Z, et al. SPIDR is required for homologous recombination during mammalian meiosis. *Nucleic Acids Res* 2023; 51: 3855–3868.

[34] Zhang Y, Li P, Liu N, Jing T, Ji Z, Yang C, et al. Novel bi-allelic variants of FANCM cause Sertoli cell-only syndrome and non-obstructive azoospermia. *Front Genet* 2021; 12: 799886.

[35] Yatsenko AN, Georgiadis AP, Röpke A, Berman AJ, Jaffe T, Olszewska M, et al. X-linked TEX11 mutations meiotic arrest, and azoospermia in infertile men. *N Engl J Med* 2015; 372: 2097–2107.

[36] Mun J-Y, Baek S-W, Park WY, Kim W-T, Kim S-K, Roh Y-G, et al. E2F1 promotes progression of bladder cancer by modulating RAD54L involved in homologous recombination repair. *Int J Mol Sci* 2020; 21: 9025.

[37] Li H, Zhuang H, Gu T, Li G, Jiang Y, Xu S, et al. RAD54L promotes progression of hepatocellular carcinoma via homologous recombination repair. *Funct Integr Genom* 2023; 23: 128.

[38] Matsuda M, Miyagawa K, Takahashi M, Fukuda T, Kataoka T, Asahara T, et al. Mutations in the RAD54 recombination gene in primary cancers. *Oncogene* 1999; 18: 3427–3430.

[39] Wesoly J, Agarwal S, Sigurdsson S, Bussen W, Van Komen S, Qin J, et al. Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis. *Mol Cell Biol* 2006; 26: 976–989.

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