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The role of DNA mismatch repair mutS/mutL homolog genes in spermatogenesis and male infertility: a systematic review and cohort study

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Abstract

Background Recent research in male infertility genetics has identified numerous candidate genes, some of which were also involved in DNA repair. Mismatch repair (MMR) genes, such as *MSH4* and *MSH5*, have been linked to male infertility due to their role in meiosis, suggesting that other MMR genes may also contribute to impaired spermatogenesis. To investigate the role of MMR genes in male infertility, we first conducted a systematic review focusing on their involvement in impaired spermatogenesis, which was followed by a multicenter cohort study assessing the occurrence of rare deleterious variants in MMR genes among men with severely impaired fertility. The present study aimed to assess the contribution of MMR genes to male infertility and to evaluate their potential clinical utility in the diagnostic workup of men with severely impaired fertility.

Methods A systematic review was conducted through a PubMed database search with a focus on the role of MMR genes in spermatogenesis. We additionally prepared a cohort study, including whole-exome sequencing data from 244 infertile men presenting azoospermia or severe oligozoospermia (< 5 million spermatozoa/ml). Rare, deleterious variants in MMR genes were classified using the ACGS Guidelines for Variant Classification 2020.

Results Following a systematic review of the literature, we gathered robust evidence supporting the strong involvement of *MSH4* and *MSH5* variants in male infertility, moderate evidence for *MLH3*, and limited evidence for other MMR genes. From our cohort, we identified likely pathogenic or pathogenic variants in two individuals: one with two *MSH4* variants and another with a *PMS2* variant.

Conclusions The present study identifies *MSH4* and *MSH5* as strong candidate genes for male infertility, supporting the integration of their testing into the clinical diagnosis of infertile men, particularly those exhibiting non-obstructive azoospermia. Although current evidence suggests that genetic variants in most MMR genes do not cause infertility,

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genetic defects in MMR genes can still impair spermatogenesis due to their critical role in sperm DNA repair and maintenance of genome integrity.

Keywords Male infertility, Spermatogenesis, Mismatch repair, MSH, MLH

Background

Male infertility is a global healthcare problem, affecting 2.5% to 12% of men [1]. Male factor is estimated to be the sole cause in 20–30% of couples, with an overall contribution of up to 50% of all infertility cases. This proportion may vary across populations, with reported estimates ranging between 20% and 70%.[1] Male infertility may result from a wide range of causes, including hormonal imbalances, chromosomal or single-gene abnormalities, and congenital defects like cryptorchidism [2–4]. Additionally, various acquired factors, such as varicocele, oxidative stress, exposure to environmental pollutants, as well as lifestyle-related factors like alcohol consumption, smoking, and obesity, have been linked with impaired fertility [2–4]. It is estimated that genetic abnormalities account for approximately 15–30% of male infertility cases, including conditions such as Klinefelter syndrome, Y chromosome microdeletions, chromosomal abnormalities (translocations, inversions, duplications, deletions), and monogenic mutations [5]. – [6] Recent advances in the genetics of male infertility have led to the identification of numerous candidate genes, suggesting that some cases of infertility may have a monogenic origin [5–7]. Currently known processes in which a pathogenic variant in a single gene may lead to spermatogenic failure include defects in genes involved in meiosis, piRNA biogenesis and function, as well as other pathways essential for germ cell development and maturation [8–10].

Since DNA repair abnormalities were associated with spermatogenesis impairment, genes coding for proteins involved with DNA repair mechanisms might have a role in male infertility [11]. One of those genes are mismatch repair (MMR) genes, which are primarily involved in correcting DNA replication errors, including small nucleotide deletions, insertions, and base pair mismatches [12]. Those proteins play a crucial role in maintaining DNA integrity, as defects in the MMR system during DNA replication were associated with increased mutagenesis and a higher risk of cancer, particularly colorectal cancer [13]. Emerging studies have suggested an additional role for MMR genes in reproduction, with *MSH4's* involvement in meiosis linking it to reproductive disorders [14–20].

To further explore the role of MMR genes in male infertility, we first conducted a systematic review of the existing literature on the involvement of other MMR genes in impaired spermatogenesis. Additionally, the occurrence of rare deleterious variants in MMR genes and their potential association with male infertility was further evaluated through a multicenter cohort study of infertile men.

Methods

Systematic literature screening

To gather the publications, a PubMed database screening was performed, using the keywords »(mismatch repair OR MMR OR mutS OR mutL OR *MSH2* OR *MSH3* OR *MSH4* OR *MSH5* OR *MSH6* OR *MLH1* OR *MLH3* OR *PMS1* OR *PMS2*) AND (male infertility OR infertility OR azoospermia OR oligozoospermia OR spermatogenesis OR meiosis OR meiotic crossing over)«. The inclusion criteria were studies related to male infertility, regardless of human or animal model studies. The publication date was restricted from the first search results from 1978 to October 11th, 2024. We excluded articles not published in the English language.

The present systematic review was prepared according to the preferred reporting items for systematic reviews and meta-analyses [21].

Study selection

We focused on publications examining the role of *MSH* and *MLH/PMS* genes in infertility, including studies on gene expression, as well as animal and human genetic studies. Reviews and articles published in languages other than English were excluded. Additionally, studies involving non-animal models (such as fungi, yeast, or plants) and publications investigating the role of MMR genes in female infertility were also excluded.

Participant selection

We included 244 men with severely impaired fertility; 171 with non-obstructive azoospermia, and 73 with severe oligozoospermia. Of the 244 infertile men, 191 were already sequenced and included in our previous study [22]. Patients were recruited based on their previous clinical data. The inclusion criteria were men with non-obstructive azoospermia or men with severe oligozoospermia (< 5 million spermatozoa/ml). Sperm concentration, age, origin, testicular volume, and FSH concentration data were obtained.

We excluded men with other known non-genetic and genetic causes for infertility, including patients with chromosomal abnormalities or patients with Y chromosome microdeletions. Additionally, we excluded men with obstructive azoospermia and men with infertility due to previous cancer treatments. We also excluded individuals presenting with clinical signs of conditions known

to impair testicular function or spermatogenesis. This included acquired or potentially reversible factors identified during diagnostic evaluation. In all cases of severe oligozoospermia, a comprehensive clinical and diagnostic assessment was performed to exclude other secondary causes that could potentially contribute to infertility.

Whole-exome sequencing and variant filtration

Whole-exome data from the 244 men with severely impaired fertility was analyzed. Whole-exome sequencing was performed as detailed in Podgrajsek et al. (2025) [22].

We focused on rare, deleterious variants in the MMR genes (*MSH2*, *MSH3*, *MSH4*, *MSH5*, *MSH6*, *MLH1*, *MLH3*, *PMS1*, *PMS2*). The population frequency of variants was set under 5% in the gnomAD exomes Database, version v2.1.1 (https://gnomad.broadinstitute.org/). Specifically, we targeted rare deleterious loss-of-function variants (frameshift, nonsense, canonical ± 1 or 2 splice site variants) and missense variants, predicted as pathogenic by the computational prediction tools (SIFT, PolyPhen-2, Mutation Taster, PROVEAN, REVEL, CADD, MetaSVM). Candidate variants were evaluated with the use of ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020 [23].

Results

Systematic literature screening

The literature screening resulted in the identification of 775 studies. Using the criteria outlined in the section 'Methods', 678 studies were excluded (Fig. 1). The analysis included 97 studies that examined the role of various MMR genes within the *MSH* and *MLH/PMS* groups in relation to spermatogenesis and their potential impact on male fertility.

Study characteristics

We included 97 studies that provided evidence for the potential involvement of *MSH* and *MLH/PMS* genes in spermatogenesis. Due to the large number of obtained studies, the tables summarizing all the obtained data are presented in The Supplementary Data (Table S1-S9). The main results are outlined in Table 1.

Rare potential disease-causing variants associated with male infertility were identified in only three (*MSH4*, *MSH5*, and *MLH3)* of the nine MMR genes. The corresponding variants and study characteristics are summarized in Table 2. Additionally, the locations of all identified variants in *MSH4* and *MSH5* were mapped onto the protein structures of *MSH4* and *MSH5*, as observed in Fig. 2.

In contrast to potential disease-causing variants, we observed single-nucleotide polymorphisms (SNPs) associated with male infertility in five (*MSH3*, *MSH5*, *MLH1*, *MLH3*, *PMS2*) of the nine genes. Although all genes were expressed in the testis, with several showing decreased expression in infertile men, only two genes (*MSH4*, *MSH5*) were notably enriched in the testis. Phenotypic differences were also observed in mouse models, with male infertility present in five genetic knockouts (*MSH4*, *MSH5*, *MLH1*, *MLH3*, *PMS2*).

Clinical data and genetic analysis

Seven patients harboring rare, potentially disease-causing variants in MMR genes were identified. We report likely pathogenic/pathogenic variants in *MSH4* and *PMS2* and potential candidate variants of uncertain significance (VUS) in *MSH5*, *MSH2*, *MLH1*, and *PMS1* (Table 3).

The clinical data of those patients is presented in Table 4.

Discussion

In the present systematic review and multicenter cohort study, we investigated potential deleterious variants in MMR genes and discussed their role in spermatogenesis and male infertility.

Based on the existing literature and our results, we propose *MSH4* and *MSH5* as genes with strong evidence for their involvement with male infertility. Mouse models for both genes confirmed the reproductive abnormalities, with disruptions leading to azoospermia and meiotic arrest at the spermatocyte stage [26–28]. These infertility phenotypes align with the functional role of *MSH4* and *MSH5*, which form a protein complex in eukaryotes responsible for binding and stabilization of Holliday junctions and facilitation of crossovers during Meiosis I, therefore ensuring chromosomal repair and segregation during meiosis [44–46]. Genetic studies on humans confirmed the involvement of genetic variants in *MSH4* and *MSH5* with infertility. In the case of *MSH4*, twelve unrelated infertile cases, and in *MSH5*, ten unrelated infertile cases with potential disease-causing variants were identified.[14–20, 39–41] In our cohort of infertile men, we identified an infertile case with two pathogenic variants in *MSH4* [22], confirming the prevalence of *MSH4* variants in the Balkan population. Regarding *MSH5*, while the variant identified in our cohort remains a VUS, it is a strong candidate for the fertility abnormalities observed in the patient. Given the numerous reported variants, including those reported in our study, alongside the strong evidence from expression and animal studies, we suggest the clinical potential of *MSH4* and *MSH5* testing for infertile men. We recommend the inclusion of *MSH4* and *MSH5* in clinical genetic panels and their implementation in the diagnosis of men with severe infertility, particularly azoospermic patients with histologically confirmed meiotic arrest.

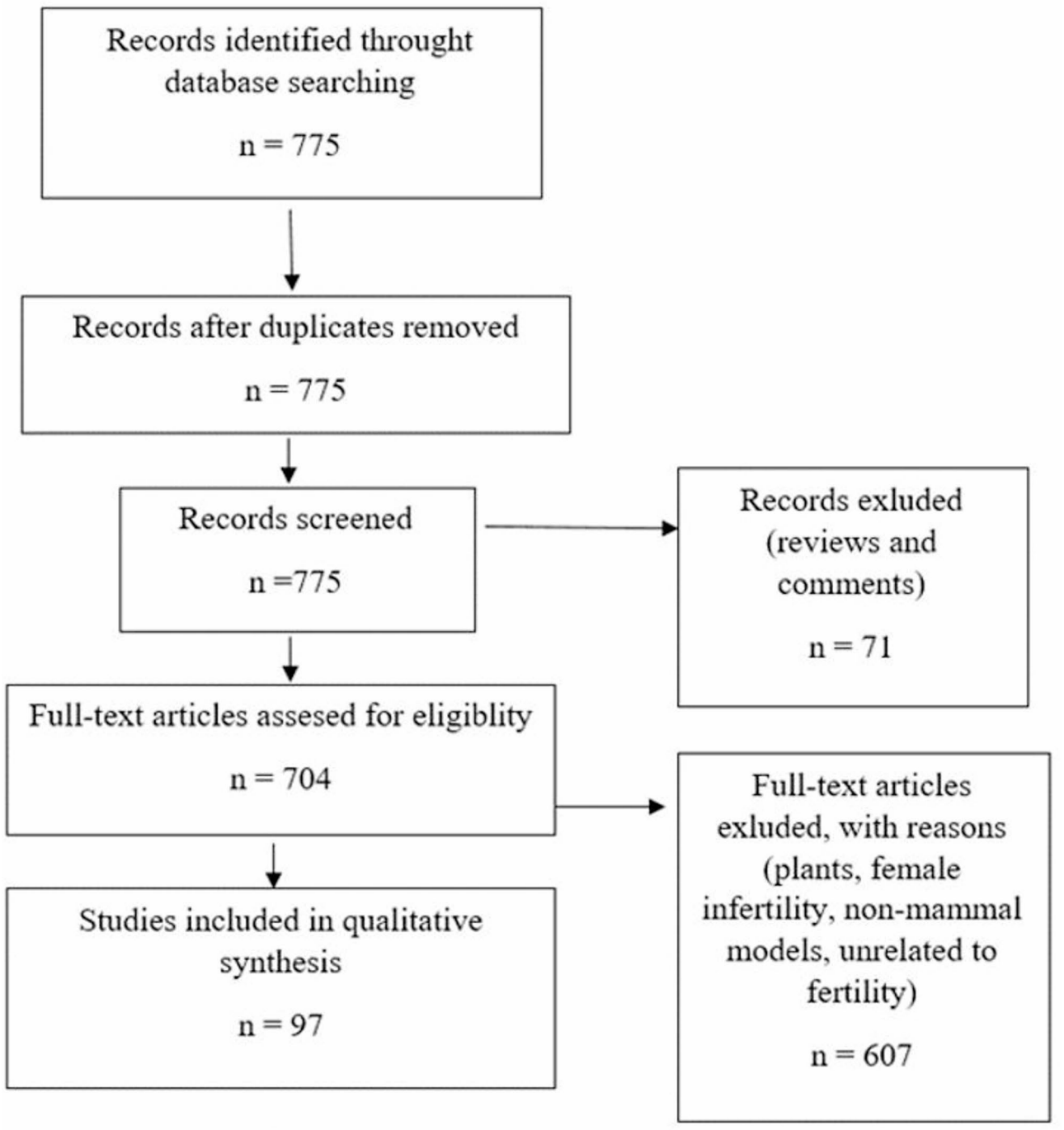

Fig. 1 PRISMA flow diagram

Besides *MSH4* and *MSH5*, mouse models additionally confirmed the potential role of other MMR genes with spermatogenesis. *MLH1*, *MLH3*, and *PMS2* knockout mice models were all infertile, [30, 35] although the infertility observed in *PMS2*-deficient mice was less severe [35]. In eukaryotes, *MLH1* and *MLH3* form a complex, which is mainly involved in meiosis rather than in MMR. According to the literature, both the *MSH3-MH4* and *MLH1-MLH3* complexes participate in the promotion of crossover formation and meiotic crossover resolution [47]. *MSH4-MSH5* heterodimer complex stimulates DNA cleavage by the *MLH1-MLH3* complex endonuclease function, which facilitates the crossing over [47, 48]. Despite the convincing evidence from the functional and animal model studies, the role of *MLH1* and *MLH3* on fertility in humans is still lacking since only two potential disease-causing variants in *MLH3* were associated with male infertility [42, 43]. Numerous SNP studies were

Table 1 Summary of the obtained evidence on mismatch repair genes with male infertility

	Number of unrelated cases with rare variants associated with male infertility	Number of SNP studies observing their association with male infertility	Expression in testis	Enriched expression in testis	Expression in infertile men	Other changes in infertile men	Mouse model -Reproductive Phenotype	Mouse model - Additional Phenotype
MSH2	-	-	+	-	Decreased	Increased sperm aneuploidy in men with *MSH2* variants. Negative correlation of promotor methylation and sperm concentration	Fertile[a]	Microsatellite instability and cancer susceptibility, increased frequency of lymphoid tumors
MSH3	-	1	+	-	-	-	-	-
MSH4	12	-	+	+	Decreased or absent	-	Infertile[b]	-
MSH5	10	5	+	+	Decreased or absent	-	Infertile[c]	-
MSH6	-	-	+	-	-	-	-	-
MLH1	-	3	+	-	Decreased or absent	Infertile men present more methylated *MLH1*. Positive correlation between *MLH1* methylation and reactive oxygen levels. Methylation of *MLH1* associated with the number of aniline blue-positive sperm	Infertile[d]	Deficient mismatch ability, microsatellite instability, susceptibility to cancer
MLH3	2	4	+	-	Decreased or absent	-	Infertile[e]	-
PMS1	-	-	+	-	Contradicting results	-	-	-
PMS2	-	1	+	-	No difference	-	Infertile/fertile[f]	Microsatellite instability, susceptibility to cancer (sarcomas and lymphomas)

[a] *Msh2* mice -/- fertile presenting some germ cell loss, apoptosis, and reduced tubule diameter [24, 25]

[b] *Msh4* mice -/- infertile (male and female), presenting azoospermia with a 50% reduction in their testis weight. Absence of development stages beyond zygonema and increased apoptosis of germ cells. Chromosome analysis revealed chromosomal pairing abnormalities during the zygotene stage, with synapsis pairing failure [26].

[c] *Msh5* mice -/- infertile (male and female) with normal sexual behavior. Mice present azoospermia with reduced testis size by 70%. Absence of normal pachytene spermatocytes. Male mice presented a meiotic arrest in the zygotene stage [27, 28] Mice with a homozygous mutation in the ATP binding domain are also infertile, however, with spermatogenesis progressing even beyond late pachynema [29].

[d] *Mlh1* mice -/- mice infertile (male and female), without detectable mature sperm. Histology revealed maturation arrest at pachytene of prophase I/late pachytene, metaphase of meiosis I. Spermatocytes presented meiotic abnormalities and apoptosis [30, 31] Mice with a homozygous mutation in the ATP binding domain are also infertile [32].

[e] *Mlh3* mice -/- infertile (male and female), presenting azoospermia. Their testes were smaller and severely depleted of spermatocytes. The majority of spermatocytes still progressed to diplonema and metaphase. Spermatocytes showed abnormal segregation, which led to aneuploidy [33]. Mice with a homozygous mutation in the endonuclease domain are infertile [34].

[f] *Pms2* mice -/-infertile (only males). The observed spermatozoa were reduced in number (< 25% of normal males) and had abnormally shaped heads with truncated flagella. Histology showed a decrease in the number of rounds and elongated spermatids. Abnormal chromosome synapsis was observed [35]. Endonuclease or ATP domain *Pms2*-deficient mice were fertile [36, 37] Mice with a homozygous variant in c.1993 A >G are fertile [38].

Table 2 Studies reporting rare sequence variants in *MSH4*, *MSH5*, and *MLH3* associated with male infertility

Gene	Phenotype	Origin of patients	Variant	Predicted protein	Zygosity	Type of variant	Number of affected infertile men	Reference
MSH4	Azoospermia	China	c.1552 C>T	p.Gln518Ter	Homozygous	Stop-gain	1	[14]
	Azoospermia	Spain	c.1913 C>T	p.Pro638Leu	Homozygous	Missense	1	[15]
	Azoospermia	Spain	c.2261 C>T	p.Ser754Leu	Homozygous	Missense	1	[15]
	Azoospermia (one with oligozoospermia)	Iran	c.2261 C>T	p. Ser754Leu	Homozygous (one heterozygous)	Missense	4 brothers	[16]
	Azoospermia	Germany	c.1453 C>T c.1686del	p.Gln485Ter p.Val563Ter	Compound heterozygous	Stop-gain	1	[17]
	Azoospermia	Netherlands	c.2198 C>A	p.Ser733Ter	Homozygous	Stop-gain	1	[17]
	Azoospermia	China	c.805_812del	p.Val269GlnfsTer15	Homozygous	Frameshift	1	[18]
	Azoospermia	China	c.1950G>A c.2179delG	p.Trp650Ter p.Asp727MetfsTer11	Compound heterozygous	Stop-gain Frameshift	2 brothers	[18]
	Azoospermia	China	c.244G>A c.670delT	p.Gly82Ser p.Leu224CysfsTer3	Compound heterozygous	Missense Frameshift	1	[18]
	Azoospermia	China	c.2220_2223del	p.Lys741ArgfsTer2	Homozygous	Frameshift	1	[18]
	Azoospermia	Iran	c.118 C>T	p.Gln40Ter	Homozygous	Stop-gain	2 brothers	[19]
	Azoospermia	China	c.2107+5G>A	/	Homozygous	Non-coding	1	[20]
MSH5	Azoospermia	Turkey	c.75dup	p.Ser26GlnfsTer42	Homozygous	Frameshift	1	[17]
	Azoospermia	Canada/Arab	c.964 C>T	p.Arg322Cys	Homozygous	Missense	1	[17]
	Azoospermia	Iraq	c.1857del	p.Ala620GlnfsTer9	Homozygous	Frameshift	1	[17]
	Azoospermia	Syria	c.1857del	p.Ala620GlnfsTer9	Homozygous	Frameshift	1	[17]
	Azoospermia	China	c.678_681del	p.Tyr227ValfsTer21	Homozygous	Frameshift	1	[39]
	Azoospermia	China	c.830 C>T c.1459G>T	p.Pro277Leu p.Asp487Tyr	Compound heterozygous	Missense	1	[39]
	Azoospermia	China	c.1459G>T c.1914 C>A	p.Asp487Tyr p.Cys638Ter	Compound heterozygous	Missense Stop-gain	1	[39]
	Azoospermia	Tunisia	c.537+1G>A	/	Homozygous	Splice donor	1	[40]
	Azoospermia	Tunisia	c.1015_2508del	/	Homozygous	Deletion (CNV)	1	[40]
	Azoospermia	Pakistan	c.1126del	p.Ser376Ala fsTer6	Homozygous	Frameshift	2 brothers	[41]
MLH3	Azoospermia	China	c.615delA	p.Asp206Thrfs*18	Homozygous	Frameshift	1	[42]
	Severe oligozoospermia	Pakistan	c.3632delA	p.Asn1211Metfs*49	Homozygous	Frameshift	2 brothers	[43]

also observed in other MMR genes, suggesting a potential role in infertility. However, despite those findings, no evidence for the association between rare sequence variants in these genes and human male infertility has yet been established. Current evidence supports the primary involvement of deleterious variants in *MLH1* and *MLH3* with cancer susceptibility and Lynch syndrome rather than male infertility [49–51].

Similarly, *PMS1* and *PMS2* deleterious variants in humans have thus far only been associated with cancer and Lynch syndrome [52, 53]. In contrast to the more evident role of *MLH1* and *MLH3* in male meiosis, the role of *PMS2* in male infertility is less clear since *PMS2* knockout mice still produced some spermatozoa [35]. Fischer et al. (2016) proposed that *PMS2's* main function is not directly related to meiosis but rather plays a supporting role by stabilizing *MLH1* levels during spermatogenesis. *PMS2* and *PMS1* knockouts led to the destabilization of *MLH1*, and the loss of *PMS2* alone was enough to destabilize *MLH1* in the testis [37]. The evidence for *PMS1's* involvement with infertility is less evident, as no mouse model could be obtained. Despite the results from the animal studies, the current evidence in humans, for now, still suggests a more established role of *MLH1*, *MLH3*, *PMS1*, and *PMS2* in cancer pathogenicity. More reproductive-focused studies should be performed to assess their involvement with impaired fertility.

Due to the lack of strong evidence for *PMS2*, *MLH1*, and *PMS1* with male infertility, the potential impact of the identified pathogenic variant in *PMS2* and the candidate VUSs in *MLH1* and *PMS1* on male infertility identified in our study could not be fully assessed. These identified variants are most likely associated with cancer susceptibility, but since the patients were not yet informed of the genetic diagnosis, the necessary diagnostic procedures to confirm this could not be performed.

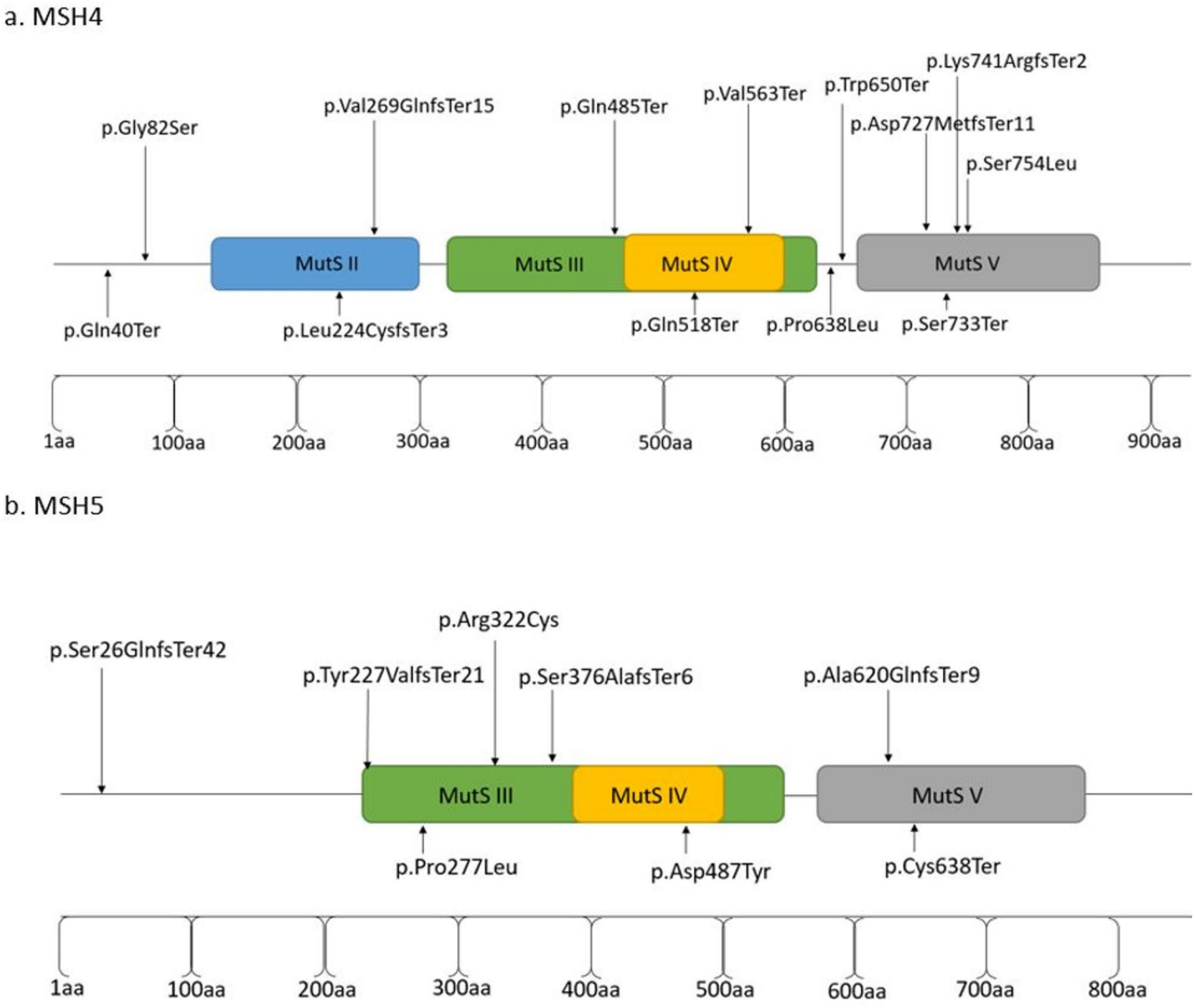

Fig. 2 Previously identified variants associated with male infertility within the domains of MSH4 (**a**) and MSH4 (**b**). The arrows indicate the position of the variant on the protein sequence (aa, aminoacid), while the domains are presented as rectangles (domain MutSII, MutSIII, MutSIV, MutSV). The figure was prepared with PowerPoint based on the data from Decipher (https://www.deciphergenomics.org/) and previous literature [14–20, 39–41].

Additionally, given that the mouse models were majority knockouts, the role of *MLH1*, *MLH3*, and *PMS2* in male infertility should not be completely dismissed. Complete loss of the protein in humans could potentially have similar effects.

Regarding other *MSH* genes (*MSH2*, *MSH3*, *MSH6*), the literature suggests their main role in MMR, as reviewed by Fishel et al. (2015) [54]. No studies observed an association between rare sequence variants in *MSH2*, *MSH3*, and *MSH6* and monogenic male infertility. However, they may still affect male fertility due to their role in DNA repair, potentially leading to compromised genomic integrity of germ cells during spermatogenesis [11]. The two variants in *MSH2* and the one in *PMS1* and *MLH1* from our study may therefore not be causative for their infertility phenotype, but could still potentially negatively contribute to fertility. The potential role of MMR genes on male infertility is further supported by SNP studies, with several SNPs in MMR genes associated with impaired fertility [55–61].

Numerous epidemiological studies have observed an increased incidence of cancer in infertile men [62]. Furthermore, although genes like *MSH2*, *MSH3*, *MLH1*, *MLH3*, *PMS1*, and *PMS2* are associated with cancer susceptibility and Lynch syndrome, common variants in those genes were observed as risk factors for male infertility [55–61]. In cancer, many processes are dysregulated, including DNA repair mechanisms. Mutations in DNA repair proteins have been implicated in tumorigenesis, as reviewed by Hopkins et al. (2022) [63]. Since proper DNA repair is also critical during spermatogenesis, abnormalities in DNA repair mechanisms could lead

Table 3 List of rare deleterious variants identified in the cohort of men with severely impaired fertility

Patient	Diagnosis	Gene	Inheritance[b]	Zygosity	Transcript change	Protein change	Variant type	ACMG criteria and outcome	Allele frequency (GnomAD exomes)
Case 1[a]	AZOO	*MSH4*	AR	Heterozygous *(de novo)*	NM_002440.4 c.1392delG	p.Ile465fs	Frameshift	PVS1, PM2, PM6 (pathogenic)	0.0000042
				Heterozygous (maternal origin)	NM_002440.4 c.2261 C >T	p.Ser754Leu	Missense	PM1,PM2, PM3, PP1_Strong, PP3 (pathogenic)	0.000028
Case 2	AZOO	*PMS2*	AD/AR	Heterozygous	NM_001322014.2 c.211_214delAATG	p.Asn71fs	Frameshift	PVS1, PS4_Moderate, PM2 (pathogenic)	/
Case 3[a]	AZOO	*MSH5*	AR	Homozygous	NM_172166.4 c.390_392delCAT	p.Ile131del	Inframe deletion	PM2, PM4_Supporting (VUS)	0.000012
Case 4	AZOO	*MSH2*	AD/AR	Heterozygous	NM_000251.3 c.662G >C	p.Gly221Ala	Missense	PM1_ Supporting, PM2, PP3 (VUS)	/
Case 5	AZOO	*MSH2*	AD/AR	Heterozygous	NM_000251.3 c.987G >C	p.Leu329Phe	Missense	PM1_ Supporting, PM2, PP3 (VUS)	/
Case 6	OAT	*MLH1*	AD/AR	Heterozygous	NM_000249.4 c.1874 A >G	p.Tyr625Cys	Missense	PM1_ Supporting, PM2, PP3 (VUS)	0.00000398
Case 7	AZOO	*PMS1*	/	Heterozygous	NM_000534.5 c.122 A >C	p.Asp41Ala	Missense	PM2, PP3 (VUS)	0.0000278

Abbreviation: *AZOO* azoospermia, *OAT* oligoasthenoteratozoospermia

[a] Variants reported in our previous study by Podgrajsek et al. (2025) [22]

[b] Inheritance was assessed using OMIM and the ClinGen Clinical Genome Resource

Table 4 Clinical data of patients with reported rare deleterious variants in mismatch repair genes

Case	Diagnosis	Sperm concentration (10^6/ml)	Age	Origin	Testicular volume (ml)	FSH (IU/L)
Case 1	AZOO	0	38	Montenegro	/	19.5
Case 2	AZOO	0	41	Montenegro	/	/
Case 3	AZOO	0	36	Serbia	L = 15 *R* = 18	1.6
Case 4	AZOO	0	30	Slovenia	L = 6 *R* = 8	19.4
Case 5	AZOO	0	32	Serbia	L = 16 *R* = 14	/
Case 6	OAT	3.8	40	Slovenia	L = 8 *R* = 8	/
Case 7	AZOO	0	31	Serbia	L = 17 D = 15	4.6

Abbreviation: *AZOO* azoospermia, *FSH* follicle-stimulating hormone, *L* left testis, *OAT* oligoasthenoteratozoospermia, *R* right testis

to spermatogenesis arrest and recombination abnormalities, negatively affecting fertility [11]. An overlap between genes involved in both cancer and spermatogenesis may account for the observed link between the etiologies. As reviewed by Nagirnaja et al. (2018), other biological processes, including genome maintenance and cell survival, could contribute to both cancer and male infertility. Given the shared biological processes, like DNA repair, abnormalities in one biological process may impact multiple disease etiologies [64]. It is therefore fundamental not to focus solely on one etiology, since spermatogenesis involves a complex network of genetic and protein interactions that overlap with those of other biological processes.

Given the association between infertility and cancer, infertile men should be monitored for potential cancer development, while cancer patients should be assessed for their potential fertility decline.

Limitations

This study has limitations that should be acknowledged. First, the interpretation of the identified variants is primarily based on in silico annotation tools and previously reported phenotypes in animal models, without functional validation. While these approaches provide valuable insights, experimental confirmation will be necessary to establish pathogenicity. Second, although we identified a pathogenic variant in *PMS2*, its broader

clinical implications regarding cancer risk in the patient were not yet assessed through follow-up screening or genetic counseling. Third, although MMR genes are known to play roles in both male and female reproduction, this study focused exclusively on male infertility and did not explore related female reproductive disorders, such as primary ovarian insufficiency. Finally, most of the newly identified variants originate from individuals of Balkan ancestry, which may limit the generalizability of our findings to other populations. Further studies in larger, ethnically diverse cohorts are needed to validate these results.

Conclusion

The present study expands the previous knowledge regarding the role of MMR genes (*MSH* and *MLH/PMS*) in male infertility. Based on previous research and the identification of a novel case with pathogenic variants in *MSH4*, we report *MSH4* and *MSH5* as genes with strong evidence for their involvement with impaired male infertility. These findings suggest that *MSH4* and *MSH5* should be considered in the clinical management and genetic testing of infertile men. Although the role of other MMR genes remains less clear, the current evidence indicates that most MMR genes, apart from *MSH4* and *MSH5*, are not related to male infertility. However, these genes may still affect fertility potential through their involvement in sperm DNA repair and the maintenance of genome integrity. Further research is required to explore the potential role of MMR genes in impaired male fertility and their possible connection to cancer.

Abbreviations

VUS	Variant of uncertain significance
MMR	Mismatch repair
SNP	Single nucleotide polymorphisms

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-025-01493-x.

Additional file 1: Supplementary Data.

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Not applicable.

Authors' contributions

R.P. was involved in the study design, variant interpretation, and manuscript writing. A.H. was involved with variant interpretation, study design, and reviewing the manuscript. A.M. was involved with the genetic analysis and bioinformatics analysis. M.S. was involved with study design and reviewing. A.A. was involved with patient inclusion and study review. O.M., M.R., I.N., D.P.K., P.N., S.O., and A.B.T. contributed the study participants' genetic material outside Slovenia and B.P. led the study design, experiments and reviewed the final version. All authors reviewed and approved the final version of the article.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (reference number: 50/03/15; 0120–213/2022/6). Prior to the start of the study, patients signed a written consent. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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