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Review Article

A Systematic Review of Genetic Causes of Male Infertility Diagnosed by Whole-Exome Sequencing in the Pakistani Population: Updates from 2015 to June 2025

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Abstract

Male infertility in Pakistan exhibits unique genetic patterns due to high consanguinity rates (65%). This systematic review of 38 studies (2015-2025) analyzed 2,041 participants (1,503 infertile men, 538 controls) using whole-exome sequencing (WES). Key findings reveal distinct genetic causes and inheritance patterns specific to this population. Chromosomal abnormalities affected 20.9% of azoospermic men, primarily Klinefelter syndrome (14.7%). Y-chromosome microdeletions occurred in 8% of cases, mostly in the AZFc region (50%). We identified 72 pathogenic variants across 58 genes, with 70.8% being novel to Pakistani populations. Consanguinity drove homozygous inheritance in 72.2% of cases. The most frequently mutated genes included ADAD2 (30% of non-obstructive azoospermia), HFM1 (20%), and DNAH family members (28.7% of motility defects). Variant types comprised frameshift (38.9%), missense (33.3%), nonsense (16.7%), and splicing mutations (11.1%). Significant biochemical markers included the CAT rs7943316 TT genotype (70.9% vs 14% controls) and elevated oxidative stress markers. These findings establish the first comprehensive genetic profile of Pakistani male infertility, demonstrating the profound impact of consanguinity on disease expression. The results emphasize the need for population-specific diagnostic protocols that prioritize DNAH/CFAP genes for motility disorders and ADAD2 for non-obstructive azoospermia. This review provides critical insights for genetic counseling and clinical management in high-consanguinity populations.

Keywords

Consanguinity Male Infertility Pakistan, Whole Exome Sequencing (WES), Male Infertility, Consanguineous Marriages, Spermatogenic Failure, Y-chromosome Deletion

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1. Introduction

Consanguineous marriages, defined as unions between biologically related individuals, represent a deeply rooted cultural practice affecting approximately 20% of the global population, particularly across South Asia, the Middle East, and North Africa [1-4]. Pakistan exhibits one of the world's highest consanguinity rates (65%), with first-cousin marriages being most prevalent, followed by India (55%) and Saudi Arabia (50%) [5]. While urbanization has gradually reduced this practice, sociocultural factors including religion, tradition, and economic considerations continue to sustain consanguinity across generations [6]. This reproductive pattern significantly impacts population genetics, increasing homozygosity for autosomal recessive disorders and contributing to elevated rates of idiopathic male infertility - estimated to be 30-40% higher in consanguineous populations compared to Western countries [7-11].

Male infertility constitutes a major global health challenge, affecting 7-12% of men worldwide and contributing to nearly 50% of cases where couples fail to conceive after 12 months of unprotected intercourse [12]. The genetic etiology is complex, with chromosomal abnormalities (e.g., Klinefelter syndrome), Y-chromosome microdeletions, and single-gene defects collectively accounting for 15-20% of cases [13-15]. Notably, spermatogenic failure remains unexplained in over 60% of azoospermic men, highlighting critical gaps in our understanding of testicular dysfunction [16]. This knowledge deficit is particularly pronounced in high-consanguinity populations like Pakistan, where unique genetic architectures may underlie infertility phenotypes.

Investigating male infertility presents substantial challenges, including phenotypic heterogeneity, the polygenic nature of spermatogenesis (involving >4,000 genes), and confounding environmental factors [17]. While next-generation sequencing has revolutionized gene discovery, studies frequently yield inconsistent results due to population stratification, small sample sizes, and variable phenotypic classifications. These limitations are especially relevant in Pakistan, where despite high infertility rates, systematic genetic studies remain scarce.

This review synthesizes a decade (2015-2025) of research on genetic causes of male infertility in Pakistan, with particular focus on whole exome sequencing findings from consanguineous families. By analyzing population-specific mutation spectra, inheritance patterns, and genotype-phenotype correlations, we aim to elucidate the unique genetic landscape of male infertility in Pakistan and identify priorities for future research and clinical translation in high-consanguinity settings.

2. Methodology

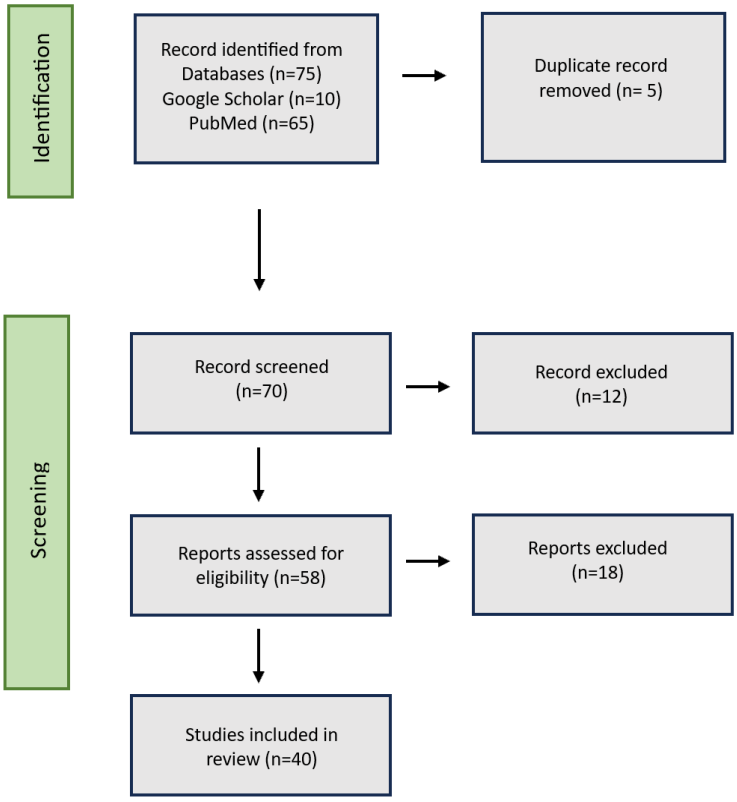

Figure 1. *PRISMA Flow Chart.*

This systematic review was conducted to analyze genetic studies on infertility disorders in Pakistani families, covering both syndromic and non-syndromic cases reported between January 2015 and June 2025 according to PRISMA guidelines. The inclusion criteria were designed to incorporate literature that provided comprehensive genetic insights into male infertility, particularly focusing on mutations, whole exome sequencing findings, and the impact of consanguinity on infertility in the Pakistani population. To achieve this objective, an extensive literature search was performed using prominent academic databases, including Web of Science, Google Scholar, and PubMed (NCBI). Relevant studies were identified using a combination of keywords such as "hereditary infertility disorders," "Pakistani mutations," "whole exome sequencing," "male infertility in Pakistan," "consanguinity and infertility in Pakistan," and related terms. The initial search results were systematically filtered to exclude irrelevant studies, ensuring that only articles directly pertinent to the genetic basis of infertility were retained. The initial search was conducted on January 4, 2025, and yielded 75 articles in total (Figure 1). Following the removal of 5 duplicates, two independent reviewers screened the remaining 70 articles against the inclusion criteria. Disagreements were resolved through discussion, leading to consensus. A total of 58 articles met the initial screening criteria and underwent further eligibility assessment. After excluding 18 articles that did not report the outcomes of interest, 40 studies were ultimately included in the systematic review.

3. Data Inclusion and Exclusion Criteria

The inclusion criteria for this systematic review were carefully defined to ensure the selection of relevant and high-quality studies. Studies were included if they met the following conditions: (1) a primary focus on consanguinity and the genetic basis of male infertility susceptibility; (2) case-control studies involving human participants, where cases consisted of infertile males with idiopathic infertility (encompassing subtypes such as Y-chromosome microdeletions, cytogenetic abnormalities, azoospermia, asthenozoospermia, teratozoospermia, and oligozoospermia) and controls were fertile males; (3) reporting of genotype or allele frequency data for both cases and controls; and (4) availability of complete full-text articles.

Conversely, studies were excluded based on the following criteria: (1) those investigating environmental factors rather than genetic causes of male infertility; (2) animal studies, review articles, meta-analyses, conference abstracts, and editorial papers; (3) duplicate publications; and (4) studies lacking relevant genetic or clinical data. These criteria ensured that only methodologically sound and directly applicable research was included in the analysis.

4. Data Extraction and Verification

A standardized approach was employed for data extraction to maintain consistency and accuracy. The following key details were retrieved from each selected study: (I) the first author's name, (II) publication year, (III) geographical region and ethnicity of the study population, (IV) genotyping methodology, (V) sample sizes for both cases and controls, (VI) sources of control groups, and [18] prevalence of genotypes and alleles.

To minimize bias, two independent authors performed the data extraction. Any discrepancies between the extracted data were resolved through discussion and, if necessary, adjudication by a third author. This rigorous verification process ensured the reliability and reproducibility of the collected data, reinforcing the validity of the subsequent meta-analysis. The extracted information was systematically organized to facilitate comparative analysis and synthesis of genetic findings across different studies.

5. Results

5.1. Chromosomal Analysis of Infertility Cases in Pakistani Population

One reported study analyzed chromosomal abnormalities in 241 individuals presenting with infertility or recurrent pregnancy loss at Liaquat National Hospital, Karachi Pakistan from 2017-2021. The cohort included 129 infertility cases (azoospermia/oligospermia) [19] and 7 recurrent pregnancy loss cases. Using standard GTG-banding techniques, we detected chromosomal abnormalities in 18.3% of participants (44/241), with higher prevalence (20.6%, 28/136). Among infertility cases, abnormalities were found in 20.9% cases. while the pregnancy loss group showed abnormalities in only in one male (1.7) 14.3%. The most common numerical abnormalities were Klinefelter syndrome (47, XXY) in males (14.7% of abnormal cases). Structural abnormalities (5.4% of total cases) included Robertsonian translocations, sex chromosome abnormalities, and heteromorphisms [20]. The detailed distribution and types of chromosomal abnormalities observed are presented in the accompanying Table 1.

Table 1. *Cytogenetic abnormalities in infertile men.*

Category	Size (N=136)
Total abnormalities	28 (20.6%)
Infertility group	27/129 (20.9%)
Miscarriage group	1/7 (14.2%)

Category	Size (N=136)
Numerical abnormalities	22 (16.2%)
Klinefelter (47, XXY)	20
Turner (45, X)	-
Structural Abnormalities	6 (4.4%)
Robertsonian translocation	1
Sex reversal (46, XX/46, XY)	1

Y-chromosome Microdeletions

The focal point of this review is the analysis of Yq microdeletions in male infertility cases in Pakistani population from 2015 to June 2025. Only one paper has been published in this duration and Yq microdeletions were detected in 12 (5.45%) of the cases, while none were found in the control group ($p \leq 0.007$). All microdeletions were identified in azoospermic men, accounting for 12 out of 150 (8.0%) cases. Among patients with microdeletions, the distribution of affected AZF regions was as follows: AZFa in 1 (8.33%), AZFb in 2 (16.67%), AZFc in 6 (50%), AZFb+c in 2 (16.67%), and complete AZF deletions in 1 (8.33%) patient [19] (Table 2).

Table 2. *Distribution of Yq microdeletions in Azoospermia men.*

Category	Number of cases (%)
Total cases with Yq microdeletions	12 (5.45%)
Microdeletions in azoospermia cases	12/150 (8%)
AZFa deletion	1 (8.33%)
AZFb deletion	2 (16.67%)
AZFc deletion	6 (50%)
AZFb+c deletion	2 (16.67%)
Complete AZF deletion	(8.33%)

5.2. Genetic Cause of Azoospermia in the Pakistani Population

5.2.1. Non-Obstructive Azoospermia (NOA)/Spermatogenic Failure

On average, men of reproductive age ejaculate approximately 96 million sperm per ejaculation [21]. Azoospermia-the complete absence of sperm in the ejaculate-affects roughly 1% of all men and 10–15% of infertile males, regardless of the underlying cause [22, 23]. In about two-thirds of azoospermic men, the condition is linked to untreatable testicular disorders leading to spermatogenic failure. Also referred to as non-obstructive azoospermia (NOA), SF represents the most severe form of male infertility [24].

Different pathogenic variants associated with non-obstructive azoospermia (NOA) and obstructive azoospermia (OA) in Pakistani families are summarized in Table 3. Overall, ten disease-causing variants across nine genes (SPATA22, MEIOB, C14orf39/SIX6OS1, MSH5, HFM1, DND1, KCTD19, ADAD2, ZSWIM7, and YTHDC2) [25-33] were identified in consanguineous Pakistani families with NOA (Table 3). Among these, ADAD2 was the most frequently mutated gene, found in three unrelated families (30%), followed by HFM1 (20%) and C14orf39/SIX6OS1 (10%). The remaining genes (SPATA22, MEIOB, MSH5, DND1, KCTD19, ZSWIM7, and YTHDC2) were each reported in a single family (10% each). All identified variants were homozygous, except for one case of compound heterozygous mutations in ADAD2. The mutations were categorized based on their type, including missense (50%), frameshift (30%), splicing (10%), and nonsense (10%) (Table 1). Functional studies revealed that these mutations disrupt critical meiotic processes, such as homologous chromosome synapsis (C14orf39/SIX6OS1, MSH5, ZSWIM7), protein-protein interactions (SPATA22-MEIOB, DND1), meiotic progression (HFM1, KCTD19, ADAD2), and RNA metabolism and translational regulation (YTHDC2).

5.2.2. Obstructive Azoospermia (OA)

One study has been reported to investigate the genetic basis of obstructive azoospermia (OA) in a non-consanguineous Pakistani family with three infertile males [34]. Comprehensive clinical assessments, including semen analysis, hormonal profiling, testicular histology, ultrasonography, karyotyping, Y-chromosome microdeletion screening, and CFTR testing, confirmed the OA diagnosis. WES of two affected brothers and two fertile relatives identified a novel nonsense variant in the X-linked ADGRG2 gene (c.2440C>T, p.Arg814*), which was prioritized as the most biologically plausible candidate. This variant, absent in ExAC and gnomAD population databases, introduces a premature stop codon predicted to truncate 204 amino acids from the critical transmembrane domain.

Table 3. *Genetic Cause of Azoospermia.*

Category	Non-Obstructive Azoospermia (NOA)	Obstructive Azoospermia (OA)
Key Genes	SPATA22, MEIOB, C14orf39, MSH5, HFM1, DND1,	ADGRG2

Category	Non-Obstructive Azoospermia (NOA)	Obstructive Azoospermia (OA)
	KCTD19, ADAD2, ZSWIM7, YTHDC2	
Frequent Mutations	ADAD2 (30%), HFM1 (20%), C14orf39 (10%)	ADGRG2 (100%)
Mutation Types	Missense (50%), Frameshift (30%), Splicing (10%), Nonsense (10%)	Nonsense (100%)
Inheritance Pattern	Primarily homozygous (90%); one compound heterozygous case (ADAD2)	X-linked recessive
Functional Impact	Disrupted meiotic synapsis (C14orf39, MSH5, ZSWIM7), impaired protein interactions (SPATA22-MEIOB, DND1), meiotic arrest (HFM1, KCTD19, ADAD2), RNA metabolism defects (YTHDC2)	Truncated ADGRG2 protein (loss of transmembrane domain)
Population Frequency	ADAD2 variants in 3 unrelated families; others in single families	Single family reported
Clinical Diagnosis	Testicular failure (high FSH, small testes)	Normal FSH, palpable vas deferens, post-testicular obstruction
Histological Findings	Sertoli cell-only, maturation arrest, hypospermatogenesis	Normal spermatogenesis with ductal obstruction
Treatment Implications	MicroTESE for sperm retrieval (30-50% success)	Surgical reconstruction or sperm retrieval (higher success)

5.2.3. Genetic and Clinical Characterization of Sperm Defects in Pakistani Families

Overall, 21 disease-causing variants across 19 genes were identified in Pakistani families from 2017 to 2025 with sperm count and motility defects. The DNAH gene family, critical for axonemal integrity, showed the highest mutational frequency (28.7%). DNAH17 [35] variants included homozygous missense (p.Ala2103Val) and nonsense (p.Gln3935*) mutations, causing severe axonemal disorganization with over 78% abnormal sperm cross-sections. DNAH1 [36] mutations featured a frameshift (p.N2549Qfs*61) and missense variant (p.C1789Y), disrupting fibrous sheath structure, while DNAH2 [37] (p.W4240C) led to asthenozoospermia with 80-90% abnormal sperm. DNAH8 [38] (p.6158_6159insT) and DNAH10 [39] (compound heterozygous p.P3137T/p.D4316H and p.G2950D/p.R3687W) were associated with MMAF phenotypes and microtubule defects.

The CFAP gene family (16.7%), essential for flagellar assembly, included CFAP43 [40] variants (p.Arg300Lysfs22, p.Thr526Serfs43) causing axonemal disassembly in 82% of sperm cross-sections, CFAP61 [41] mutations (p.I151Nfs*4, p.R283*) leading to absent central pairs, and CFAP57 [42] loss-of-function variants (p.R958, p.R913) contributing to MMAF. SPAG17 [43] mutations (p.Asp212_Glu276del, p.Leu707) resulted in incomplete C1a projections and missing microtubule doublets. ARMC variants collectively contributed 16.7% of cases; ARMC2 [44] (p.S61X) and ARMC3 [45] (p.Glu245_Asp305delfs16) variants disrupted central pair complexes and mitochondrial sheath organization, respectively.

Additional genes implicated in diverse sperm defects (38.8% frequency) included NPHP4 [46] (p.P497R), associated with a 9+0 microtubule configuration; TTC12 [47] (p.Arg357Trp), causing flagellar ultrastructural defects; and CCDC34 [48] (p.A283E), linked to outer dynein arm absence. AK7 [49] splicing variants (c.871-4ACA>A) led to disorganized axonemes and abnormal mitochondrial sheets, while ADCY10 [50] mutations (p.Arg968*, splicing c.4286+1G>T, c.436+2T>G) misassembled mitochondrial sheaths. TBC1D25 [51] (p.Glu50Ala) impaired autophagy-related processes, STK33 [52] (p.T412Kfs*14) caused complete flagellar disorganization, and ACTL7A [53] (p.E50Afs*6) showed a founder effect in Pashtun descendants with acrosomal detachment. Notably, a novel homozygous variant in ENO4 [54] [c.293A>G, p.(Lys98Arg)] was identified in two consanguineous brothers with severe sperm head defects, including globular, pyriform, and elongated morphologies with reduced head size, despite normal karyotypes (46 XY) and absent Y-chromosome microdeletions.

Reported genes in the Pakistani population exhibit distinct mutational patterns and phenotypic consequences. Frameshift mutations predominated (38.9%), followed by missense (33.3%), nonsense (16.7%), and splicing mutations (11.1%). Consanguinity profoundly influenced inheritance patterns, with 72.2% of variants occurring in homozygous state, while compound heterozygous variants accounted for 22.2%. A single X-linked recessive variant (5.6%) was identified in TBC1D25 (p.Glu50Ala). Functional categorization showed 44.4% of variants caused axonemal disassembly, 27.8% led to central pair complex defects, 16.7% resulted in fibrous sheath abnormalities, and 11.1% affected mitochondrial organization.

Different pathogenic variants associated with male infertility are summarized in Table 4.

Table 4. *Different Pathogenic Variants Associated with Sperm Motility and Defects.*

Gene	Variant	Mutation Type	Zygosity	Phenotypic Impact	Structural Defects	Frequency
DNAH17	c.6308C>T (p.Ala2103Val) c.11803C>T (p.Gln3935*) c.G5408A (p.C1803Y)	Missense Nonsense Missense	Homozygous Homozygous Homozygous	Severe axonemal disorganization	≥78% abnormal cross-sections, disrupted 9+2 array	14.3%
DNAH1	c.7646_7647insC (p.N2549Qfs*61) c.6212T>G (p.C1789Y)	Frameshift Missense	Homozygous Homozygous	Fibrous sheath defects	Missing central singlets, disorganized FS	9.5%
DNAH2	c.12720G>T (p.W4240C)	Missense	Homozygous	Asthenozoospermia	80-90% abnormal sperm morphology	4.8%
DNAH8	c.6158_6159insT	Frameshift	Homozygous	MMAF	Divergent flagellar ultrastructure	4.8%
DNAH10	c.9409C>A (p.P3137T) c.12946G>C (p.D4316H) c.8849G>A (p.G2950D) c.11509C>T (p.R3687W)	Missense Missense Missense Missense	Compound Heterozygous	MMAF	Microtubule defects, head abnormalities	4.8%
CFAP43	c.900_901del (p.Arg300Lysfs*22) c.1576_1577del (p.Thr526Serfs*43)	Frameshift Frameshift	Compound Heterozygous Homozygous	MMAF	82% abnormal cross-sections, absent CPC	9.5%
CFAP61	c.451_452del (p.I151Nfs*4) c.847C>T (p.R283*)	Frameshift Nonsense	Homozygous Homozygous	MMAF	Missing central pairs, absent RS/IDA	4.8%
CFAP57	c.2872C>T (p.R958*) c.2737C>T (p.R913*)	Nonsense Nonsense	Homozygous Homozygous	MMAF	Flagellar assembly defects	4.8%
SPAG17	c.829+1G>T (p.Asp212_Glu276del) c.2120del (p.Leu707*)	Splicing Frameshift	Homozygous Homozygous	CPC defects	Complete CPC absence	4.8%
ARMC2	c.182C>G (p.S61X)	Nonsense	Homozygous	Mitochondrial defects	Vacuolated mitochondria, disrupted flagella	9.5%
ARMC3	c.916+1G>A (p.Glu245_Asp305delfs*16)	Splicing	Homozygous	MMAF	Incomplete C1a, missing doublets 1/9	9.5%
NPHP4	c.1490C>G (p.P497R)	Missense	Homozygous	9+0 configuration	Absent central microtubules	4.8%
TTC12	c.1069C>T (p.Arg357Trp)	Missense	Homozygous	Flagellar defects	Ultrastructural abnormalities	4.8%
CCDC34	c.848C>A (p.A283E)	Missense	Homozygous	OAT	Missing outer dynein arms	4.8%
AK7	c.871-4ACA>A	Splicing	Homozygous	MMAF	Disorganized axonemes, abnormal mitochondria	4.8%
ADCY10	c.2902C>T (p.Arg968*) c.4286+1G>T c.436+2T>G	Nonsense Splicing Splicing	Compound Heterozygous Homozygous	Midpiece defects	Misarranged mitochondrial sheaths	4.8%

Gene	Variant	Mutation Type	Zygosity	Phenotypic Impact	Structural Defects	Frequency
TBC1D25	c.197A>C (p.Glu50Ala)	Missense	X-linked	Oligozoospermia	Autophagy impairment	4.8%
STK33	c.1235del (p.T412Kfs*14)	Frameshift	Homozygous	MMAF	Complete flagellar disorganization	4.8%
ACTL7A	c.149_150del (p.E50Afs*6)	Frameshift	Homozygous	Acrosomal defects	98.9% acrosomal detachment (founder variant)	4.8%
ENO4	c.293A>G (p.Lys98Arg)	Missense	Homozygous	Sperm head defects	Globular/pyriform heads, reduced size	4.8%

5.3. Association of MTHFR Polymorphisms with Male Infertility

The association of MTHFR polymorphisms [55] (C677T: rs1801133 and A1298C: rs1801131) with male infertility was investigated in Pakistani populations across two studies. In the first study (346 participants: 232 infertile, 114 fertile), the C677T variant showed distinct genotypic distributions: 72.8% of infertile men were homozygous (CC), 22.8% heterozygous (CT), and 4.3% homozygous for the risk allele (TT), compared to 86.8% (CC), 10.5% (CT), and 2.6% (TT) in controls. While the homozygous minor (TT) genotype did not differ significantly, the C allele frequency was significantly higher in controls ($\chi^2 = 8.22$; OR = 2.17; $p = 0.004$). For A1298C, infertile men exhibited a higher frequency of the homozygous risk genotype (CC: 19.0% vs. 2.8% in controls), with statistically significant genotypic ($\chi^2 = 16.47$; OR = 8.28; $p < 0.0001$) and allelic differences ($\chi^2 = 18.24$; OR = 2.09; $p < 0.0001$).

A second study (437 infertile men: 57 azoospermic, 66 oligospermic, 44 asthenozoospermic, 29 teratozoospermic, 20 oligoasthenoteratospermic [OAT], 221 normospermic infertile; 218 fertile controls) confirmed the MTHFR C677T [56] association. The minor 677T allele significantly increased infertility risk ($p < 0.05$), with CT (OR: 1.81, 95% CI: 1.17-2.80; $p = 0.008$) and TT (OR: 9.24, 95% CI: 1.20-70.92; $p = 0.032$) genotypes showing elevated risk. All infertility subgroups had higher CT/TT frequencies versus controls ($p < 0.05$), and combined CT + TT genotypes were significantly associated with infertility (OR: 2.01, 95% CI: 1.31–3.08; $p < 0.001$).

5.4. Molecular and Endocrine Markers of Male Infertility in the Pakistani Population

Recent studies on male infertility in Pakistani populations have revealed several key molecular and endocrine markers that distinguish fertile from infertile men. Research has demonstrated significant oxidative stress imbalances, with infertile men showing markedly reduced catalase (CAT) enzyme activity (0.20 ±0.01 U/mg) compared to fertile controls (0.33 ±0.03 U/mg). This oxidative imbalance was particularly pronounced in men with the CAT rs7943316 TT genotype, which appeared in 70.9% of infertile cases versus only 14% of controls. Concurrently, elevated levels of superoxide dismutase [57] and reduced glutathione (GSH) were observed across all infertility subtypes, suggesting a compensatory response to oxidative damage [58].

Further investigations into neuroendocrine factors identified elevated acetylcholinesterase (AChE) activity in infertile men, along with a significant association of the ACHE rs17228602 variant with infertility risk [59]. These findings were accompanied by increased levels of the pro-inflammatory cytokine IL-1β, indicating an inflammatory component in male reproductive dysfunction. Endocrine profiling of azoospermic patients revealed distinct patterns, with non-obstructive azoospermia (NOA) cases showing elevated follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone, and estradiol levels, but significantly reduced kisspeptin compared to obstructive cases [60].

At the mitochondrial level, studies demonstrated downregulation of sirtuin genes (SIRT3, SIRT4, SIRT5) in infertile men, along with an abnormal inverse relationship with HSP90 expression [61]. Genetic analyses identified androgen receptor CAG repeat length as another important factor, with longer repeats significantly associated with spermatogenic defects [62]. These findings collectively paint a picture of male infertility as a multifactorial condition involving oxidative stress, endocrine dysregulation, mitochondrial dysfunction, and genetic predisposition, providing valuable insights for both diagnosis and potential therapeutic interventions in the Pakistani population.

6. Discussion

This systematic review presents a comprehensive analysis of genetic causes of male infertility in the Pakistani popula-

tion diagnosed through whole exome sequencing (WES). The high rate of consanguinity (65%) in Pakistan has significantly influenced the genetic architecture of male infertility, with 72.2% of identified variants occurring in homozygous states. This finding aligns with the country's tradition of endogamous marriages and highlights the advantage of using WES combined with homozygosity mapping in consanguineous populations.

Our analysis revealed that 70.8% of the variants associated with male infertility in Pakistan were novel, underscoring the unique genetic landscape of this population. The remaining 29.2% of variants had been previously reported in other populations, demonstrating both shared and population-specific genetic contributions to infertility. Among the most frequently mutated genes were ADAD2 (identified in 30% of NOA cases) and HFM1 (20%), followed by C14orf39/SIX6OS1 (10%). The DNAH gene family emerged as particularly significant, accounting for 28.7% of sperm motility defects, with DNAH17 variants being the most prevalent.

Non-obstructive azoospermia (NOA) was the most common phenotype (66.7% of azoospermia cases), primarily caused by mutations in meiotic genes such as SPATA22, MEIOB, and MSH5. In contrast, obstructive azoospermia (OA) was less frequent and predominantly linked to the X-linked ADGRG2 gene. The predominance of NOA reflects the severe impact of consanguinity on spermatogenic failure, as most causative variants were autosomal recessive.

Frameshift mutations (38.9%) and missense variants (33.3%) were the most common mutation types, followed by nonsense (16.7%) and splicing mutations (11.1%). These genetic defects primarily disrupted critical biological processes including meiotic synapsis (C14orf39/SIX6OS1, MSH5, ZSWIM7), axonemal assembly (DNAH family, CFAP genes), and mitochondrial organization (ARMC2, ARMC3). The high frequency of DNAH and CFAP family mutations (45.4% combined) in sperm motility defects suggests these genes should be prioritized in genetic screening panels for Pakistani men with asthenozoospermia.

Our findings demonstrate that male infertility in Pakistan has distinct genetic patterns compared to other populations. The high prevalence of novel variants and the predominance of autosomal recessive inheritance reflect the effects of consanguinity.

Abbreviations

AChE	Acetylcholinesterase
AZF	Azoospermia Factor (regions a, b, c)
CAT	Catalase
CFAP	Cilia and Flagella Associated Protein
CI	Confidence Interval
CPC	Central Pair Complex
FS	Fibrous Sheath
FSH	Follicle-Stimulating Hormone
GTG-banding	G-banding using Trypsin and Giemsa
GSH	Glutathione
HSP90	Heat Shock Protein 90
IL-1β	Interleukin-1 beta
LH	Luteinizing Hormone
MMAF	Multiple Morphological Abnormalities of the Flagella
MTHFR	Methylenetetrahydrofolate Reductase
NOA	Non-Obstructive Azoospermia

Author Contributions

Musavir Abbas: Writing Original Draft

Nisar Ahmad: Conceptualization, Data curation, Formal Analysis, Methodology, Visualization, Writing – original draft

Ahmad Faraz: Formal Analysis

Manahil Younas: Formal Analysis

Zain-Ul-Abideen: Formal Analysis

Wasim Shah: Conceptualization, Formal Analysis, Investigation, Project administration, Supervision, Validation, Writing – review & editing

Data and Code Accessibility

The authors confirm that all the data are already presented in the article.

Approval for Publication

After reviewing the manuscript, the authors have decided to submit it to the publication. The authors declare that nothing in the study has ever been published before or is presently being considered for publication anywhere.

Ethics and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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