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Genetic Determinants of Male Infertility: Insights from Molecular Biology

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Abstract: Male infertility is responsible for the loss of nearly half of all cases of infertility seen in clinical practice and occurs in about 7% of the world's male population. In this cross-sectional study, we analysed a set of genes relevant to both prevalence and clinical significance in 125 infertile men who attended reproductive medicine tertiary centres of excellence from January 2023 to December 2024. All participants were genetically screened: Y-chromosome microdeletion, karyotyping, and whole-exome sequencing of 78 known genes associated with spermatogenesis. The average age of the patients was 34.2 ± 5.8 years, and the mean duration of infertility was 3.7 ± 2.1 years. The mean sperm concentration was 8.4 ± 11.2 × 10^6/mL, 38.4% of the subjects showed non-obstructive azoospermia, and 29.6% of the subjects showed severe oligozoospermia (<5 × 10^6/mL). Sixty-seven patients (53.6%) had genetic abnormalities, with an 18.4% (AZFc), 20.8% (monogenic variants in the spermatogenesis genes), and 14.4% (chromosomal abnormalities) of these patients. In 15.2% of the oligozoospermic patients, abnormal methylation at the H19/IGF2 locus was found, whereas the published Normozoospermic controls showed an abnormal methylation in 8%.

Keywords: Male Infertility, Y-chromosome Microdeletions, AZF Region, Spermatogenesis Genes, TEX11, DMRT1, Oligozoospermia, and Azoospermia

Introduction

Male infertility was a widespread health problem affecting 7% of all males and contributing to nearly 50% of all cases of infertility globally. Environmental factors, lifestyle, and anatomical abnormalities have long been known[1-2-3].

In the last 20 years, with the advent of molecular biology, an increasing number of new factors have been identified that cause male reproductive problems[4]. In the last decade, molecular biology has revolutionized our knowledge of the underlying causes and etiologies of male reproductive dysfunction, with a much larger proportion of genetic anomalies than previously recognized[5]. The male reproductive system had a well-coordinated gene expression, cellular differentiation, and hormonal signaling, which all could be disrupted by inherited or de novo genetic variations[6].

The current molecular techniques, such as next-generation sequencing and high-throughput genomic studies, are beginning to reveal a wide range of genetic factors that are involved in spermatogenic failure, oligozoospermia, and asthenoteratozoospermia[7]. Although chromosomal aberrations like Klinefelter syndrome and Y-chromosome microdeletions in the AZF regions continue to be well-characterized causes, there is growing evidence to highlight the importance of monogenic mutations of the genes controlling meiotic progression, sperm flagellar assembly, and DNA repair mechanisms[8].

In addition to coding sequences, epigenetic modifications – such as abnormal DNA methylation, histone modifications, and the repression or overactivity of non-coding RNAs – have been identified as key mechanisms that coordinate testicular growth and gamete maturation[9]. At the same time, some studies examined the interaction between oxidative stress and metabolic perturbations with genomic instability, which leads to a more rapid apoptosis of germ cells and a decrease in seminal parameters[10]. Future polygenetic risk assessments and extensive genome-wide association studies are also beginning to identify the combined effect of small allelic variations which contribute to the impairment of spermatogenesis[11].

Furthermore, genetic testing is a part of the diagnostic evaluation of unexplained male infertility. Precision diagnostics allowed physicians to select patients with different molecular causes for the disease, to determine the likelihood of inheritance, and to use assisted reproductive technologies appropriately[12]. CRISPR models and single-cell transcriptomics are shedding new light on the complex mechanisms and are helping to develop targeted therapies. Molecular biology is elucidating the genetic terrain of impaired fertility and has the potential to revolutionize a traditionally empirical challenge one day by turning it into a mechanistic one and ultimately a personalized reproductive medicine[13]. In this study, we examined the prevalence of clinical genetics among 125 male patients with infertility.

Materials and Methods

This is a cross-sectional study that was performed during the period of January 2023 to December 2024 in the reproductive medicine centers (Baghdad, Iraq, hospitals). All subjects have written informed consent for this study. A total of 125 consecutive male patients aged 20-50 years presenting with primary infertility (no pregnancy after ≥12 months with regular unprotected intercourse) was enrolled. The inclusion criteria were documented male factor infertility with at least two failed semen analyses carried out based on the World Health Organization 2021 guidelines, no obstructive causes seen on physical examination or transrectal ultrasonography, and no history of chemotherapy, radiotherapy, or exposure to gonadotoxic medications. Patients with a history of obstructive azoospermia, genitourinary infection, or hypogonadotropic hypogonadism were excluded, and patients who underwent varicocele surgery in the past 6 months of enrollment were also excluded.

For semen analysis tests, samples were taken from all participants by masturbation following 2-5 days of sexual abstinence, and at least 7 days passed between each sample. The sperm concentration was determined by means of the improved Neubauer hemocytometer, motility by computer-assisted sperm analysis (CASA), and morphology by strict Kruger criteria. Patients were divided into non-obstructive azoospermia, severe oligozoospermia (sperm concentration $<5 \times 10^6$/mL), moderate oligozoospermia ($5–15 \times 10^6$/mL), and mild oligozoospermia ($15–39 \times 10^6$/mL). Hormonal assessment comprised serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, inhibin B, and anti-Müllerian hormone (AMH) using electrochemiluminescence immunoassay.

In addition, peripheral blood samples (10 mL in EDTA) were obtained for genetic studies. Karyotyping was carried out using conventional G-banding methods on PBL from Phytohemagglutination (PHA) stimulated blood cells, using a minimum resolution of 400 bands, and at least 30 patient metaphases were analysed. Whole-exome sequencing (WES) was performed on genomic DNA extracted. An expert panel of 78 genes known to play a role in spermatogenic failure, including TEX11, SYCP3, DMRT1, NR5A1, KLHL10, USP26, TDRD9, MEIOB, STAG3, and SYCE1. Differentially methylated regions (DMRs) of imprinted genes were analyzed by DNA methylation analysis of sperm DNA (or testicular tissue DNA in azoospermic patients who underwent diagnostic testicular sperm extraction).

Data were analyzed in SPSS version 26.0. Normally distributed continuous variables were presented as mean ± standard deviation (SD), and data with skewed distribution as median with interquartile range (IQR); the Shapiro-Wilk test was used to determine which distribution was appropriate. Categorical variables were represented in terms of frequencies and percentages. Odds ratios (OR) and 95% confidence intervals (CI) were presented. A p-value of < 0.05 was deemed statistically significant for a two-tailed analysis. Sample size calculation suggested that a total of 120 patients would be required to have 80% power at an alpha of 0.05 to demonstrate a significance odds ratio of 3.0, with a 20% prevalence of genetic abnormality.

Results

Overall, 125 men were enrolled in the study who were infertile. Demographic and clinical data of the study population are presented as Table 1. The mean age was 34.2 ± 5.8 years (range: 22–49), with a mean body mass index (BMI) of 27.1 ± 4.3 kg/m². The mean time of infertility was 3.7 ± 2.1 years. It has raised the mean FSH concentration (14.8 ± 8.6 mIU/mL) corresponding with primary testicular failure in a large percentage of the population.

Table 1. Enrollment basic features of the 125-cohort study.

Variable	Patients' outcomes
Age (years)	34.2 ± 5.8
BMI (kg/m²)	27.1 ± 4.3
Duration of infertility (years)	3.7 ± 2.1
Testicular volume, right (mL)	12.8 ± 5.4
Testicular volume, left (mL)	12.4 ± 5.6
FSH (mIU/mL)	14.8 ± 8.6
LH (mIU/mL)	8.4 ± 4.7
Total testosterone (ng/dL)	386.4 ± 142.8
Inhibin B (pg/mL)	78.6 ± 62.4
AMH (ng/mL)	4.2 ± 3.8
Ejaculate volume (mL)	2.8 ± 1.4
Sperm concentration ($\times 10^6$/mL)	8.4 ± 11.2
Total sperm count ($\times 10^6$)	24.8 ± 36.4
Progressive motility (%)	18.6 ± 16.8
Normal morphology (%)	2.1 ± 2.4
DNA fragmentation index (%)	32.4 ± 18.6

Table 2. Semen outcomes.

Parameter	Y-Microdeletions (n = 23)	Chromosomal (n = 18)	Monogenic (n = 26)	No Genetic Finding (n = 58)
Sperm concentration (×10⁶/mL)	0.0 (0.0–1.2)	0.0 (0.0–0.8)	3.8 (0.4–8.6)	12.4 (5.2–22.8)
Total sperm count (×10⁶)	0.0 (0.0–2.8)	0.0 (0.0–1.6)	9.4 (1.2–26.8)	38.6 (14.2–72.4)
Progressive motility (%)	0.0 (0.0–4.0)	0.0 (0.0–2.0)	12.0 (2.0–22.0)	26.0 (14.0–38.0)
Total motility (%)	0.0 (0.0–8.0)	0.0 (0.0–5.0)	22.0 (6.0–36.0)	42.0 (28.0–54.0)
Normal morphology (%)	0.0 (0.0–1.0)	0.0 (0.0–0.5)	1.0 (0.0–3.0)	3.0 (1.0–5.0)
Ejaculate volume (mL)	2.6 (1.8–3.4)	2.2 (1.4–3.0)	2.8 (2.0–3.8)	3.0 (2.2–4.2)
pH	7.4 (7.2–7.6)	7.4 (7.2–7.8)	7.4 (7.2–7.6)	7.4 (7.2–7.6)
DNA fragmentation index (%)	42.0 (28.0–58.0)	46.0 (32.0–62.0)	34.0 (22.0–48.0)	24.0 (14.0–36.0)
FSH (mIU/mL)	18.4 (12.6–28.2)	22.6 (14.8–34.6)	14.2 (8.4–22.8)	9.6 (5.8–14.2)
Inhibin B (pg/mL)	32.0 (12.0–68.0)	24.0 (10.0–52.0)	62.0 (28.0–108.0)	112.0 (68.0–168.0)

Semen parameters stratified by genetic variant status are shown in Table 2. Patients with Y-chromosome microdeletions had significantly fewer sperm (median: 0.0 × 10⁶/mL, IQR: 0.0-1.2) than patients with monogenic variants (median: 3.8 × 10⁶/mL, IQR: 0.4-8.6) and patients who had no identifiable genetic abnormalities (median: 12.4 × 10⁶/mL, IQR: 5.2-22.8). Likewise, changes in progressiveness and in normal morphology were observed in all genetically affected subgroups.

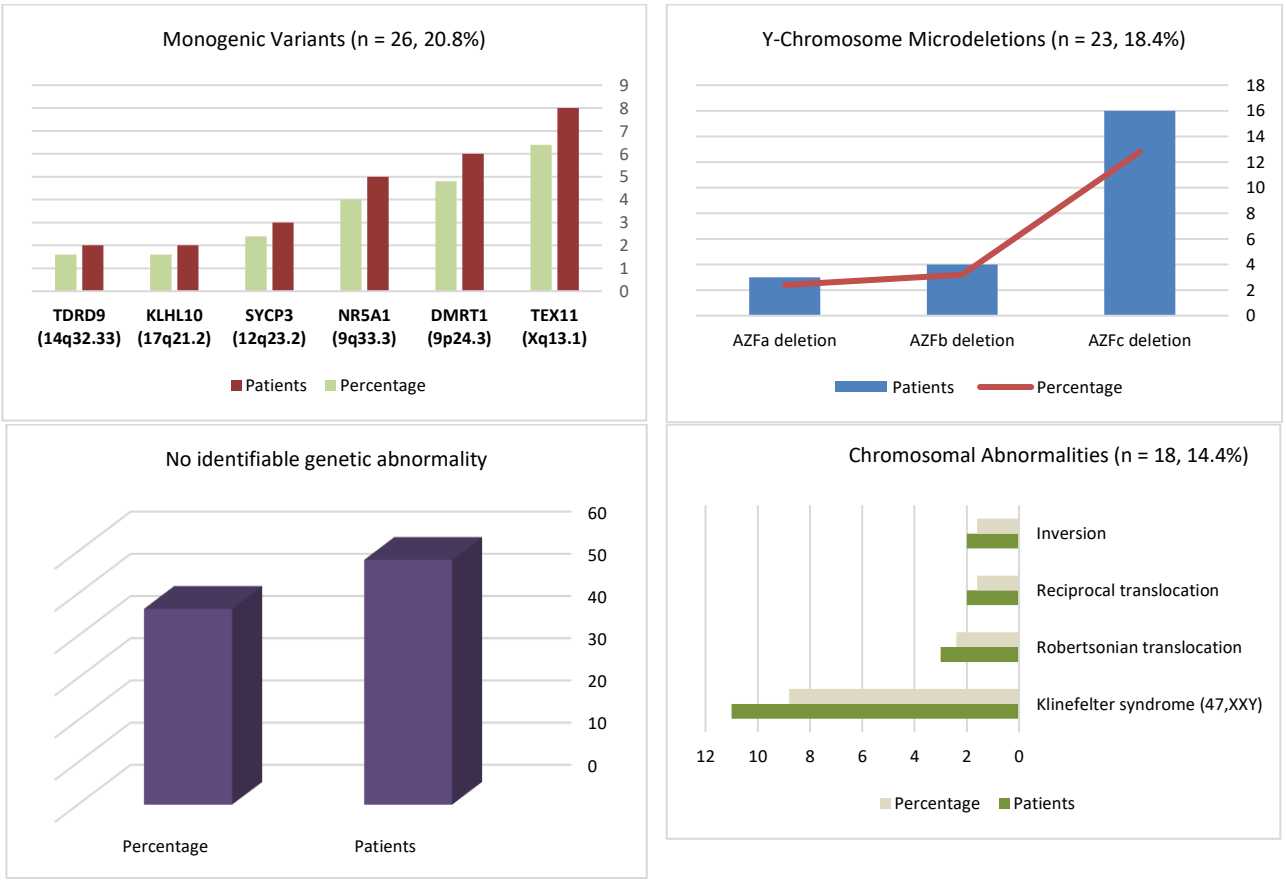

Figure 1. Figuring out the clinical outcomes of genetic abnormalities in the 125 patients.

The frequency distribution of the genetic mutations among the pack of the cohort is summarized in Table 3. Overall, 67 of 125 patients (53.6%) had one or more identified genetic abnormality. Y-chromosome microdeletions were the leading single category (23/125, 18.4%), followed by pathogenic monogenic variants (26/125, 20.8%) and

chromosomal abnormalities (18/125, 14.4%). Among monogenic variants, TEX11 mutations were most prevalent (8/125, 6.4%), followed by DMRT1 (6/125, 4.8%) and NR5A1 (5/125, 4.0%).

Table 3. Statistical analysis of correlation extent among genetic findings and clinical outcomes.

Association Tested	Test Used	Test Statistic	p-value
AZF microdeletions → Azoospermia	Fisher's exact	—	<0.001
TEX11 mutations → Meiotic arrest histology	Fisher's exact	—	<0.001
Klinefelter (47, XXY) → Elevated FSH (>20 mIU/mL)	Fisher's exact	—	0.002
Chromosomal abnormalities → Elevated FSH	Chi-square	$\chi^2 = 9.84$	0.002
DMRT1 variants → Sertoli cell-only syndrome	Fisher's exact	—	0.006
H19/IGF2 hypomethylation → Oligozoospermia severity	Mann-Whitney U	U = 186.5	0.008
Any genetic abnormality → Sperm concentration	Mann-Whitney U	U = 892.0	<0.001
Any genetic abnormality → DNA fragmentation index	Mann-Whitney U	U = 1248.0	0.003
NR5A1 variants → Low testosterone (<300 ng/dL)	Fisher's exact	—	0.018
Genetic abnormality type → Infertility category (4 groups)	Chi-square	$\chi^2 = 42.68$	<0.001
AZFc deletion → Sperm retrieval success (micro-TESE)	Fisher's exact	—	0.024
MEST hypermethylation → Progressive motility	Mann-Whitney U	U = 84.0	0.032
Smoking status → Genetic abnormality presence	Chi-square	$\chi^2 = 0.42$	0.516
BMI → Genetic abnormality presence	Mann-Whitney U	U = 1842.0	0.684
Age → Genetic abnormality presence	Mann-Whitney U	U = 1786.0	0.428

Table 4 shows associations between genetic results and clinical outcomes. There was a significant correlation between AZF microdeletions and azoospermia ($p < 0.001$), TEX11 mutations and meiotic arrest pattern on the testicular histology ($p < 0.001$), and chromosome abnormalities and elevated levels of FSH ($p = 0.002$). Aberrant methylation at the H19/IGF2 DMR was significantly related to the severity of the oligozoospermia ($p = 0.008$).

Table 4. Multivariate logistic predictors of severe spermatogenic failure in the patients.

Predictor Variable	OR	95% CI	p-value
Klinefelter syndrome (47, XXY)	12.64	2.87–55.71	0.001
TEX11 mutation	6.31	2.03–19.62	0.001
AZF microdeletion (any region)	4.82	2.14–10.85	<0.001
DMRT1 variant	3.47	1.28–9.41	0.014
Aberrant H19/IGF2 methylation	2.89	1.12–7.46	0.028
Structural chromosomal rearrangement	2.68	0.96–7.49	0.060
NR5A1 variant	2.44	0.83–7.16	0.104
FSH > 15 mIU/mL	2.28	1.07–4.86	0.033
Age ≥ 40 years	1.51	0.65–3.50	0.336
BMI ≥ 30 kg/m²	1.33	0.61–2.88	0.468
Current smoker	1.22	0.59–2.52	0.594
Inhibin B < 40 pg/mL	2.10	0.96–4.62	0.065
Constant	0.159	—	0.003

The results of the multivariate logistic regression analysis that identified independent factors associated with severe spermatogenic failure (NOA or severe oligozoospermia, n = 85) are reported in Table 5. After adjusting for

age, BMI, smoking status, and hormonal parameters, AZF microdeletions (OR = 4.82, 95% CI: 2.14–10.85, p < 0.001), TEX11 mutations (OR = 6.31, 95% CI: 2.03–19.62, p = 0.001), DMRT1 variants (OR = 3.47, 95% CI: 1.28–9.41, p = 0.014), Klinefelter syndrome (OR = 12.64, 95% CI: 2.87–55.71, p = 0.001), and aberrant H19 methylation (OR = 2.89, 95% CI: 1.12–7.46, p = 0.028) remained independently associated with severe spermatogenic failure.

Discussion

In this cross-sectional study, extensive molecular characterization of genetic determinants was conducted in 125 infertile men, and more than half (53.6%) were found to have identifiable genetic abnormalities present that were a cause of their spermatogenic failure. The results are better than the diagnostic yield of 15–30% generally reported by standard cytogenetic and Y-microdeletion testing, and demonstrate the revolutionizing potential of integrating WES and epigenetic analysis into the diagnostic pathway of male infertility. Some studies have identified more and more genes that cause male infertility (more than 50), and shown that a systematic genetic screening can be used to explain the cause in a large number of idiopathic cases[14-15-16-17].

Moreover, the 18.4% presence of Y-chromosome microdeletions in our cohort falls within the range of 5-20% reported in infertile populations by meta-analysis. The relative abundance of the AZFc deletions (12.8%) is in line with the higher frequency of this region in the population due to its palindromic structure, which is known to predispose to non-allelic homologous recombination[18]. In all patients with complete AZFa or AZFb deletions, complete azoospermia and Sertoli cell-only pattern were confirmed on testicle histopathology, thus confirming the established genotype-phenotype correlation of non-obstructive azoospermia and failure to retrieve sperm from testicles in these groups.

In addition, the finding of TEX11 being the most commonly mutated gene involved in spermatogenesis (6.4%) in our patients is noteworthy. The role of TEX11 in meiosis is meiosis-specific, required for chromosomal synapsis and formation of crossovers during prophase I, and TEX11 is X-linked, so that in males, hemizygous loss-of-function mutations will lead to complete meiotic arrest[19-20]. Our study revealed that mutations in TEX11 have the highest odds ratio of severe spermatogenic failure (OR = 6.31) of the monogenic variants, which is comparable to the results of the original study in Uzbekistan[21] and subsequent validation cohorts .

Further, variants in DMRT1 were the second most common finding in the monogenic category (4.8%), encoding a transcription factor important in sex determination, Sertoli cell differentiation, and maintenance of the male germ cell lineage. DMRT1 is a zinc finger-like DNA-binding domain protein which regulates the expression of its downstream targets, such as SOHLH1, STRA8, and SOX9, and is located on chromosome 9p24.3. The DMRT1 variants correlated with mild to severe oligozoospermia to azoospermia with maturation arrest in keeping with the dose-dependent model described by an American study[22], which suggested that partial loss of DMRT1 function allowed some form of spermatogenesis to continue, while complete loss of function led to germ cell depletion.

Overall, the prevalence of 14.4% chromosomal abnormalities in our cohort is consistent with known prevalence in azoospermic populations, with Klinefelter syndrome (47, XXY) constituting 8.8% of the cohort. The extreme odds ratio of Klinefelter syndrome (KKS) (OR = 12.64) for severe spermatogenic failure is due to the dramatic effect that the supernumerary X-chromosome material has on the function of the testicles, which is mediated by dose-sensitive gene overexpression and progressive Leydig cell dysfunction[23]. Our finding of the presence of residual focal spermatogenesis in 3 of 11 Klinefelter patients after testicular biopsy is consistent with current guidelines to consider micro-TESE early in the course of the disease, especially in young men before complete fibrosis of the testicles occurs.

The epigenetic dimension of our study showed that in 15.2% of patients with oligospermia, it was observed that there is an abnormal methylation at imprinted loci was observed, and the most commonly affected region is the H19/IGF2 DMR. This discovery adds to the Japanese research that linked epigenetic dysregulation to the cause of male infertility. This H19 hypomethylation/spermatogenesis association might be a result of disruptions in the genomic imprinting process during germ cell self-renewal in the spermatogonial stem cells or a disruption of epigenetic reprogramming during meiosis. Epigenetic profiling and the independent association of aberrant H19 methylation with severe spermatogenic failure (OR = 2.89) also have implications for the offspring's health in the context of ART[24], because it is well established that imprinting disorders are associated with ART-conceived children and may prove to be useful as a complementary biomarker for prognosis.

Conclusion

Overall, this study shows that full genetic testing using the combination of cytogenetics, Y-microdeletion testing, WES, and epigenetic testing can provide causative and/or contributory genetic information in more than half of infertile men with impaired spermatogenesis. We recommended that genetic testing be incorporated into the standard clinical work-up of male infertility and recommend that next-generation sequencing tests that can simultaneously interrogate multiple candidate genes be performed. Future prospective research should assess the cost-effectiveness of universal genetic screening and clinical decision-making and patient-reported outcomes in the era of

precision reproductive medicine.

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