Infertility

Role of Genetics in Azoospermia

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OBJECTIVE
To review established genetic causes of azoospermia, the most severe form of male infertility, and help clinicians, scientists, and infertile couples considering assisted reproductive technologies (ART) to understand the complexity of the disorder and to maximize the chances of having a healthy infant through proper counseling and treatment.

METHOD
An initial literature search was performed on PubMed using the key words “azoospermia” “oligospermia,” and “genetics.” The results were limited to the studies on humans and written in English, which were written within last 10 years. Although preliminary query results showed more than 900 articles, further queries using key words, such as “Y chromosome,” “monogenics,” “aneuploidy,” “mitochondrial DNA,” and “epigenetics,” along with “azoospermia,” narrowed the results to 30 papers, which were included in the present study.

RESULTS
Genetic defects causing azoospermia were categorized into two large categories: chromosomal and nonchromosomal. Chromosomal defects were further categorized into (1) structural abnormalities, such as Y chromosome micro/macrodeletions, chromosomal inversions, and translocations; and (2) numerical abnormalities, also known as aneuploidy. Nonchromosomal defects included sperm mitochondrial genome defects and epigenetic alterations of genome.

CONCLUSIONS
As a result of advancements in ART, understanding the potential implications of genetic disorders for infertile couples is critical. Analysis of a potential genetic role in azoospermia holds promise to expand our knowledge to evaluate male infertility and to guide treatments.


Infertility is defined as “a failure to conceive after 12 months of unprotected sexual intercourse.” Male factor is responsible for 30% to 50% of cases of infertility, and as many as 20% of infertile men are diagnosed as azoospermic. Azoospermia affects 1% of the male population. Some conditions directly cause azoospermia, whereas others are the result of complex gene-environment interactions.

Although advancements in reproductive medicine, such as intracytoplasmic sperm injection (ICSI) and microsurgical testicular sperm extraction (micro-TESE) allow men with minimal spermatogenesis to successfully reproduce, genetic defects may be passed down from sperm. The present study summarizes contemporary knowledge of the role of genetic disorders in azoospermic males.

EVALUATION OF AZOOSPERMIA

Diagnosis of Azoospermia
A thorough sexual and medical history, hormonal measurements, and physical examination of external genitalia are the key components of evaluation. In 70% of examined men, this preliminary evaluation will identify the cause. The remaining 30% of men require additional evaluation, such as genetic testing to elucidate the underlying cause.

Classification of Azoospermia
Azoospermia is classified as (1) obstructive azoospermia (OA) caused by obstruction of the ejaculatory pathway and (2) nonobstructive azoospermia (NOA) caused by failure of spermatogenesis. Depending on the etiology, it may also be pretesticular, testicular, or post-testicular.

Various checkpoints in spermatogenesis are controlled by a multitude of genes and signaling pathways that regulate meiosis, mitosis, and sperm transport. OA is associated with a congenital bilateral absence of the vas deferens (CBAVD), inflammation, and obstruction, which cause physical barriers for normal spermatozoal transit. NOA may be associated with either intrinsic testicular defects (also known as primary testicular failure) or hypothalamic-pituitary-adrenal axis abnormalities (also known as secondary testicular failure). The two different etiologies are easily distinguished based on hormone levels.

GENETIC CAUSES OF AZOOSPERMIA
Approximately 29% of azoospermic men have underlying genetic abnormalities. Common genetic abnormalities
include chromosomal or gene defects (nuclear or mitochondrial), and epigenetic alterations.

**Chromosomal Disorders.** Approximately 4% of males undergoing ICSI have chromosomal abnormalities—80% involve sex chromosomes. Robertsonian translocations and Klinefelter syndrome (KFS) are the most prevalent chromosomal disorders affecting 10% to 20% of azoospermic men, which will be discussed later in this manuscript.

Chromosomal disorders may involve sex chromosomes or autosomes. Numerical sex chromosomal aneuploidies are more commonly observed in azoospermic men.

**Y Chromosome Microdeletion.** Y chromosome microdeletions are found in 5% to 15% of infertile men with azoospermia and are the most common cause of azoospermia.

The region critical for germ cell development and differentiation on Y chromosome long arm (Yq) is the azoospermic factor region (AZF). The AZF region encompasses multiple gene families, AZFa, AZFb, and AZFc. AZFc is the most commonly found (60%) deletion compared with AZFa (5%), AZFb (16%), or combined (14%). This is in part because the length of AZFc is four times longer than AZFa.

Men with deletions spanning more than one AZF loci are usually azoospermic, as are men who harbor an AZFa and AZFb deletion. AZFc deletions are usually associated with a variable phenotype caused by the presence of autosomal homologue and multiple copies. Luddi et al. (2009) reported that a deletion of the USP9Y gene on AZFc, caused both azoospermia and severe oligospermia.

Studies have shown that men with an AZFc deletion experience a steady decline in sperm count and, over a period, develop azoospermia. Men with an AZF deletion are at an increased risk of losing the Y chromosome because of Y chromosome instability.

**Aneuploidy.** KFS is the most frequent cause of sex chromosome aneuploidy and leads to NOA. Approximately 14% of azoospermic males have KFS. Approximately 95% of men with KFS have 47,XXY chromosomal complement. KFS men have reduced testis size, elevated follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, as well as low testosterone (T) levels. As much as 69% of men with KFS can have successful surgical sperm retrieval via micro-Tese.

Although as much as 69% of men with KFS can have successful surgical sperm retrieval via micro-Tese, approximately 80% of 45,X/46,XY individuals have a normal SRY gene, approximately 60% of these individuals present with mixed gonadal dysgenesis with a streak gonad and a testis, a uterus, and a vagina. This karyotype is usually seen in cases harboring AZF deletions, where, because of chromosome instability, the Y chromosome is lost, resulting in mosaicism. Researchers have hypothesized that cytogenetic mosaicism or mutations in SRY downstream tests—determining genes, such as Wilms’ tumor suppressor, DAX1, and testatin (20p11.2) may play a role in the gonadal failure in these men.

**X Chromosome–Linked Abnormalities.** The role of genes on the X chromosome in male infertility remains mostly unknown, partly because researchers have relied on rodent studies, which may not accurately reflect human expression patterns. Thus, the clinical relevance is limited to less than 10 genes, such as the androgen receptor gene (AR), which is present in both human and mice.

Studies on these genes remain inconclusive, X chromosome’s hemizygotic nature in males suggests that de novo X chromosome mutations have a definite impact.

TEX11 (Xq13.1) and TAF7L (Xq22.1) play critical roles in the promotion of synthesis and regulation of crossovers. The disruption of these genes causes segregation defects and azoospermia.

The most widely studied X chromosome–linked gene is AR Xq11.2-12. Because the AR mediates the actions of androgens, mutation of the AR gene manifests various androgen insensitivity phenotypes ranging from 46,XY infertile female to 46, XY azoospermic males. In the transactivation domain (exon 1) of the AR gene, 2 polymorphic triplet repeats—(CAG)n and (GGC)n—are present. Although some studies have found an inverse correlation between CAG repeat length and male infertility, others have not, suggesting that further investigations should examine both significantly longer and/or shorter CAG repeats and their functionality.

Kallmann syndrome (KS) is a common infertility disorder that occurs in approximately 1 in 30,000 live births. About 11% of KS men have X chromosome–linked gene mutations, whereas others show autosomal inheritance pattern. KS is characterized by decreased secretion of gonadotrophin-releasing hormone during embryonic development caused by a deletion in the KAL-1 gene on X chromosome (Xp22.3). Low levels of sex steroid hormones, especially androgen, hinder normal spermatogenesis, leading to azoospermia and underdeveloped male genitalia in KS.

**Autosomal Abnormality.** Robertsonian translocations (RTs) are the most frequent structural chromosomal ab-
Normalcy. They occur 1 in 1000 live births. Reciprocal and RTs are nine times more common in infertile men than in healthy males. Recent studies have found hot spots for translocation in 265 infertile men at loci 1p31-33, 3p21.1-9, 7q31, Xq28, and 6p21, 6p22. Some of these are testis-specific genes and others may represent novel loci, which may play a role in the regulation of testicular function.

Chromosomal inversions in autosomes 1, 3, 4, 6, 9, 10, and 21 are more common in infertile men. Inversions on chromosome 9 associated with azoospermia are eight times more common in infertile men.

Cystic fibrosis (CF) is an autosomal-recessive disorder caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene (7q31.20). About 90% of men with CF have OA caused by CBAVD. The CFTR gene encodes for a CFTR protein that plays an important role in sodium/chloride balance in epithelial secretion regulated by cAMP. CF manifests with varying phenotype from vasal agenesis to full-spectrum CF symptoms. The delta F508 mutation is the most common mutation (50–80%) on the CFTR gene.

Nonchromosomal Disorders: Epigenetic Alterations
Abnormal DNA methylation and histone modification affect normal embryogenesis via transcriptional control and can ultimately cause azoospermia. Aberrant histone modifications in male infertility has not been thoroughly explored; the involvement of DNA methyltransferases and methyl-binding domain proteins play critical roles in the methylation of germ cells. Moreover, of its close proximity to the electron transport chain and its lack of histones and introns. Approximately 85% of sperm contains mtDNA deletions, and many of them have two to seven deletions, which increase with age and oxidative stress. Although multiple mtDNA deletions in azoospermic males have been detected, recent studies have found that the incidence of mtDNA deletions are not significant enough to be differentiated, but that the size of the deletions were larger in azoospermic males than in fertile males.

Nonchromosomal Disorders: mt Genome
Sperm mtDNA provides energy for spermatogenesis and sperm motility. mtDNA is prone to mutations because of its close proximity to the electron transport chain and its lack of histones and introns. Approximately 85% of sperm contains mtDNA deletions, and many of them have two to seven deletions, which increase with age and oxidative stress. Although multiple mtDNA deletions in azoospermic males have been detected, recent studies have found that the incidence of mtDNA deletions are not significant enough to be differentiated, but that the size of the deletions were larger in azoospermic males than in fertile males.

Management and Prognosis
OA
Men with CBAVD are successfully treated with TESE. For CBAVD men, it is assumed they harbor a CFTR mutation. The partner should also be screened for 51 common CFTR mutations if mutations are found; the siblings of the CFTR male have a 50% chance of being a carrier.

NOA
Men with KS have a complete lack of testosterone and do not undergo puberty. Treatment in such men requires replacement therapy with LH analogues until serum T levels are normal. FSH analogue is then started. After 12 to 24 months of treatment, these men go through puberty and have sperm in their ejaculate.

In men with KFS, T treatment helps to develop secondary sexual characteristics but does not initiate spermatogenesis. In mosaic KFS, sperm may be retrieved with micro-Tese but FISH should be considered to prevent aneuploidy.

Men with large AZF deletions and deletions involving AZFa and AZFb loci present with azoospermia and have poor prognosis on ART. However, men harboring an AZFc deletion may have adequate sperm found by TESE and can undergo ART after comprehensive counseling about their risk of similar or even larger deletions in their male offspring.

Men harboring an AZFc microdeletion should undergo sperm cryopreservation. Men with large AZF deletions are also at risk of losing the Y chromosome during fertilization. In such cases, offspring will exhibit a 45, XO karyotype or will be mosaic and may have sexual ambiguity.

Men harboring mtDNA nucleotide variations usually have high levels of reactive oxygen species (ROS), which leads to mt dysfunction. High ROS levels damage sperm nuclear and mtDNA. Thus, early diagnosis and prompt antioxidant treatment in such men may prevent irreversible DNA damage and help maintain adenosine triphosphate levels to sustain spermatogenesis.

Genetics is one of the most important yet underemphasized cause of azoospermia. Improved understanding of the genetics of infertility holds promise to define the etiology of azoospermia and counsel cases that were previously diagnosed with idiopathic azoospermia.
CONCLUSIONS
The clinical implications and frequency of certain genetic defects associated with azoospermia enable us to screen for specific genetic abnormalities. Men with OA or NOA will commonly have identifiable genetic abnormalities, and genetic counseling and treatment should be implemented and aimed at optimizing patient outcome.

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References