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.”1 Male factor is responsible for 30% to 50% of cases of infertility, and as many as 20% of infertile men are diagnosed as azoospermic.2 Azoospermia affects 1% of the male population. Some conditions directly cause azoospermia, whereas others are the result of complex gene environment interactions.3

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.3 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.2 In 70% of examined men, this preliminary evaluation will identify the cause.2 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.4 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.5 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.6,7

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.8 AZFc is the most commonly found (60%) deletion compared with AZFa (5%), AZFb (16%), or combined (14%).9 This is in part because the length of AZFc is four times longer than AZFa.9

Men with deletions spanning more than one AZF loci are usually azoospermic, as are men who harbor an AZFa and AZFb deletion.10 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.11

Studies have shown that men with an AZFc deletion experience a steady decline in sperm count and, over a period, develop azoospermia.12 Men with an AZF deletion are at an increased risk of losing the Y chromosome because of Y chromosome instability.12

**Aneuploidy.** KFS is the most frequent cause of sex chromosome aneuploidy and leads to NOA. Approximately 14% of azoospermic males have KFS.13 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.7 Although retrieval spermatozoa are usually haploid, there have been reports of KFS in offspring, a risk that can be greatly minimized with embryo screening using preimplantation genetic diagnosis (PGD) and fluorescence in situ hybridization (FISH).14

The 46,XX male syndrome is a rare aneuploidy that occurs in 1 in 20,000 live births. Approximately 90% of these males have sex determining region on the Y chromosome (SRY) translocated to the X chromosome or an autosome.3 Although 46,XX males exhibit normal inter-
Some of these are spots for translocation in 265 infertile men at loci 1p31-33, 3p21.1-9, 7q31, Xq28, and 6p21, 6p22.1 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, because of its high activity in the adult testis, the methylation status of the promoter region of the methyltetrahydrofolate reductase (MTHFR) gene has been investigated and abnormal hypermethylation in the CpG island within the promoter region was detected in males with NOA, whereas the same region was unmethylated in the controls. Thus, the hypermethylated promoter region may down-regulate MTHFR enzymatic activities, resulting in azoospermia. It has also been reported that retrieval of germ cells from testes may result in the collection of epigenetically immature germ cells.

The expression profiles of micro-RNAs, noncoding RNAs have been studied to investigate the correlation between miRNA aberrant expression and their involvement in spermatogenesis. Because 5’ and 3’ untranslated regions of mRNA are regulated by miRNAs, they may play an important role in post-transcriptional control of gene expression. The role sperm miRNAs play in the control of gene expression is very probable, given their presence in the early embryonic stages.

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


12. Dada R, Gupta NP, Kucheria K. Semen cryopreservation in 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.


