The genetics of male reproductive failure: What every clinician needs to know

What do we know about the genetic causes of reproductive failure in men who are otherwise healthy? What is the rationale for testing? What therapeutic and counseling interventions are most appropriate, based on the genetic diagnoses? Research and clinical experience are beginning to provide some answers.

Two conditions are commonly encountered in the evaluation of the infertile male: non-obstructive azoospermia (NOA) and congenital bilateral absence of the vas deferens (CBAVD). Different genetic backgrounds influence the incidence of these abnormalities. It is important to recognize them in order to properly counsel and treat both the patient and his partner.

NONOBSTRUCTIVE AZOOSPERMIA

NOA is clinically diagnosed when the ejaculate volume is > 1.5 cc, the seminal fluid pH is alkaline (both of these indicating normal seminal vesicles and ejaculatory ducts), and the reproductive ductal structures are palpably normal. The testes are typically smaller than average and may be softer in consistency compared with testes with adequate sperm production capability. Since the germ cell compartment comprises most of the bulk of the testicular tissue, the size of the testis may be a direct reflection of markedly reduced spermatogenesis.

There is a notion that the follicle-stimulating hormone (FSH) value must be exceptionally high—e.g., above 2–3 times the upper limit—for spermatogenic failure to be conclusively diagnosed. This is incorrect. The value of FSH in men with perfectly adequate spermatogenesis is usually in the lower aspect of the reference range for any particular assay. When spermatogenic capability is compromised, the pituitary compensates with an increased secretion of FSH. Therefore, in the face of azoospermia, no evidence of an obstructive process on history or physical examination, and an FSH value above the lower limits of the reference range, spermatogenic compromise is highly likely (Figure 1). If, for example, the reference range for an assay is

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KEY POINTS

- Nonobstructive azoospermia is diagnosed when the ejaculate volume is > 1.5 cc, the seminal fluid pH is alkaline, and the reproductive ductal structures are palpably normal.
- 15% to 20% of men with nonobstructive azoospermia have deletions of the Y chromosome or karyotypic anomalies.
- Before any operative intervention or use of sperm for ICSI, a Y chromosome microdeletion assay and karyotype should be performed in any male with severe oligospermia or non-obstructive azoospermia.
- Congenital bilateral absence of the vas deferens is associated with mutations within the cystic fibrosis (CF) gene in 70% to 80% of men. Thus, all men who present with vasal agenesis require a CF mutation analysis prior to intervention.
- Proper genetic testing and counseling is necessary prior to moving forward with interventions such as microsurgical epididymal sperm aspiration or testis sperm extraction.
- Patients deserve to be educated about the genetic basis for their reproductive failure.
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2–20 mIU/mL, and the FSH value of an azoospermic patient is 9, it is likely to be this high because of compensatory output by the pituitary in response to a suboptimal spermatogenic compartment within the testis.

Always be sure to centrifuge the semen specimen, as the pellet may show motile or non-motile individual spermatozoa that were not seen on routine analysis. Occasionally there are enough sperm that intracytoplasmic sperm injection (ICSI) can be carried out. If there are no sperm in a centrifuged pellet, testicular sperm extraction (TESE) may recover sperm in approximately 50% of NOA cases.

Identifying a genetic cause
Because so many men with significant compromise of the spermatogenic axis may be candidates for ICSI, it is of great importance to identify a genetic basis if possible. Both a Y chromosome microdeletion assay and a karyotype should be performed prior to any intervention. Not only may these tests be prognostic, they also provide information the couple can use to refine and define their reproductive plans.

As a reproductive clinician, I try not to speculate on what a given patient might choose to do or what tests might be helpful. Rather, I offer all the information I can to educate the patient and partner so they can make the best decision they can. Their outlook may occasionally be quite different from what we may have expected. Reproduction with one’s own gametes is a choice and not a mandate. Not all couples choose to use their own gametes when their offspring may be affected by the genetic aberrations they carry.

Microdeletion of genetic regions
The Y chromosome consists of a short arm and a long arm. The ends of the short and long arms are known as pseudoautosomal regions and pair with the X chromosome during mitosis and meiosis. The intervening large segment of the Y chromosome, known as the Male Specific Y (MSY, Figure 2), contains many genes that are involved in spermatogenesis. Two regions of the MSY in particular are prone to microdeletion, AZFa and AZFc.

Microdeletion of the AZFa region is found in slightly less than 1% of men with NOA. These patients are highly unlikely to have sperm found upon TESE, therefore TESE is not required. Without this genetic information, most patients would likely proceed with TESE in hopes of finding spermatozoa that could be used for an ICSI cycle. However, a patient who knows that he has an AZFa microdeletion—with little, if any, chance of finding sperm—may decide not to subject himself to a surgical intervention that has little hope of success.

Microdeletion also occurs in the AZFc region (Figure 3). An AZFc microdeletion occurs in 1 in 4,000 men and is the most common molecular cause of NOA. These men are somatically healthy and have no increased incidence of cryptorchidism or testicular cancer. Serum FSH, testosterone, and luteinizing hormone are not predictive of an AZFc microdeletion, nor are physical findings. Key facts:

- Approximately 70% of men with an AZFc microdeletion will possess sperm, either in the ejaculate or testis tissue.
- 13% percent of men with NOA will be AZFc microdeleted.
- Approximately 6% of men with severe oligospermia, < 5 million/ cc, will be AZFc microdeleted, thus ICSI should not be offered prior to testing in these couples.

Avoiding unneeded interventions
It is not true that “because we have sperm to use that is all that matters.” If spermatozoa are
present in the ejaculate or testis tissue in a man with an AZFc microdeletion, their quality is normal and fertilization, embryo development, and live birth are all eminently possible. The children born are somatically healthy, and the female offspring should be reproductively fit. However, male offspring will necessarily inherit the AZFc microdelet ed Y chromosome from their father, and they would be expected to show the same spectrum of spermatogenic deficiency found in our present AZFc-microdeleted population. These sons may not show the same level of spermatogenesis their father possessed (Figure 4). When they reach adulthood, our best guess is that they will either be severely oligospermic, azoospermic with sperm within the testis tissue, or azoospermic without sperm in the testis tissue. This latter group, at this time, is considered sterile.

Not all men with an AZFc microdeletion proceed to ICSI if they have sperm available for use. Some decide not to use their own sperm and choose an alternative route. Once more, this is a good example of why a Y chromosomal microdeletion assay should be performed prior to intervention in men with severe oligospermia or NOA. It is the couple’s choice as to whether they pass along severe infertility or sterility to their male children. In addition, it is possible and reasonable that preimplantation genetic screening could be used to transfer only female embryos and avoid the reproductive consequences that a male child will necessarily experience.

Another microdeletion, initially identified as AZFb, simply represents an expanse of DNA in the P5 and P1 palindromes (Figure 3). Evidence continues to accumulate that men with a P5/P1 palindrome microdeletion will not have sperm within the ejaculate or testis tissue. In a patient with NOA, these microdeletions are quite prognostic, and a very reasonable response would be to not pursue TESE. In programs where TESE and ICSI are always coordinated and combined events where TESE is performed on the same day as oocyte recovery, a P5/P1 microdeletion will save both partners intensive interventions when there is no hope that spermatozoa will be present to be placed with those oocytes. This is yet another example of why it is critical to perform a Y chromosomal microdeletion assay prior to any intervention in a patient with NOA.

**Karyotypic anomalies**

**Klinefelter syndrome.** A peripheral karyotype demonstrates the number and structure of that person’s somatic chromosomal complement. The most common abnormality found in patients with NOA is that of an extra X chromosome, 47, XXY or Klinefelter syndrome (Figure 5). This syndrome is found in approximately 1 in 500 males and is the result of non-disjunction of the X chromosome during meiosis. One of the two parental gametes, sperm or egg, carries the additional X chromosome.

The classic description of the Klinefelter male is that of a teenager who fails to undergo puberty due to lack of virilization. These males are on the severe end of the phenotypic spectrum. Both their spermatogenic and androgenic axes have failed completely. These boys are unlikely to have spermatogenic capability, as their testes are small, the seminiferous tubules sclerotic, and the Leydig cells hypertrophied and nonfunctional.

There is an opposite end of this severe phenotypic spectrum, and it includes men who may present for infertility in their 20s or 30s and are diagnosed in the course of their infertility: Treatable with ICSI Sterility

<Figure 3. A portion of the Y chromosome contains palindromes P5 through P1 and the AZFb and AZFc subregions. When illegitimate homologous recombination occurs between two sequences, such as b2 and b4, all of the intervening chromosomal material (distal P3, all of P2, and the majority of P1) is lost. This deleted region is termed AZFc; males with this microdeletion may have minimal levels of spermatogenesis. Longer spans of microdeletion may also occur via the same mechanism (P5/proximal P1 or P5/distal P1). Patients with either of these microdeletions will have a complete absence of spermatogenesis, obviating the need for testicular sperm extraction. MSY, Male Specific Y.>

<Figure 4. The predicted range of outcomes is shown for the sons of a male with the AZFc microdeletion of the Y chromosome in whom there are available sperm. Their reproductive spectrum will be significantly compromised and not necessarily the same as their father’s was. TESE, testicular sperm extraction.>
workup. They have escaped detection of their genetics because they virilized adequately during puberty, and their testosterone levels are biologically sufficient to fuel normal libido and erectile capability. Thus, there really is no classic Klinefelter presentation but rather, as with all genetic disorders, a wide phenotypic spectrum.

Approximately 50% of those with Klinefelter syndrome who present as adults will have sperm found upon TESE. The sperm can serve as the source of the male gamete to establish pregnancy with ICSI. There is a slight chance of transmission, and some clinicians believe that preimplantation genetic diagnosis should be offered during the ICSI cycle. However, this risk is contrary to what has been seen clinically where all babies born to date have had a normal 46, XX or 46, XY chromosomal constitution. Many of these men, while having biologically adequate levels of testosterone, are at risk of requiring testosterone replacement subsequent to TESE. The remaining Leydig cells may not be able to boost their testosterone production to maintain adequate levels.

Males with Klinefelter syndrome are at greater risk of breast cancer and osteoporosis than non-Klinefelter males. The diagnosis is thus important not only for the immediate reproductive discussion but also for the patient’s long-term general health. It is not enough to focus only on whether we can or cannot obtain sperm for use with ICSI; we also must try to understand and decipher the medical or genetic condition that may have led to reproductive compromise. We are physicians and not just sperm/egg technicians and harvesters.

46, XX male syndrome occurs when a small piece of the most distal aspect of the short arm of the Y chromosome is present somewhere in the genome. This small piece may contain the gene SRY that is a critical cog in the cascade of genes that determines in which direction the primitive gonad will develop. If SRY is present, the bipotential gonad develops along testicular lines. The phenotype of the 46, XX male patient will be male. However, he is missing the entire MSY, and the prediction for sperm production is dismal.

To date, 46, XX males have been found to possess no level of spermatogenesis within harvested testicular tissue. A karyotype of 46, XX male syndrome is completely prognostic. This result absolutely predicts that TESE will not be successful, and, therefore, it is critically important to provide this diagnosis to the patient prior to surgical intervention.

Isodicentric Y chromosome may be found in a small number of men with NOA. It is comprised of two short arms, two centromeres, and a small portion of the proximal long arm. Distal to that point, all Y material has been lost. It is important to recognize an isodicentric Y and to characterize which regions no longer exist. For example, if the isodicentric point occurs proximal to the P5 palindrome, the prognosis for the presence of sperm would be the same as that for a patient with a P5/P1 microdeletion—i.e., the patient will not have sperm within the testis tissue. If the isodicentric point is within the AZFc region or distal to it, the patient may have usable spermatozoa.

The isodicentric Y chromosome, however, is considered unstable and may be lost during cell replication and division. The male offspring may have cell lines that are devoid of a Y chromosome. This may result in phenotypic abnormalities such as those found in mixed gonadal dysgenesis—once again a rationale for testing prior to intervention.

Ring Y chromosomes, truncated Y chromosomes, and other autosomal abnormalities, such as translocations, are also found in a small percentage of men with spermatogenic failure (Figure 5). Translocations occur in 1% to 3% of males with NOA. It is important to recognize a translocation prior to intervention with ICSI. Preimplantation genetic screening may enhance that couple’s ability to achieve a healthy term pregnancy by allowing transfer of only normal or chromosomally balanced embryos.

The take-home message: A Y chromosome microdeletion assay and karyotype should be performed in any male with severe
oligospermia or non-obstructive azoospermia prior to operative intervention or use of the sperm for ICSI. These tests can avoid unnecessary interventions and help couples make informed choices.

**CONGENITAL BILATERAL ABSENCE OF THE VAS DEFERENS**

CBAVD is found in approximately 1% of infertile males and is one of the more common diagnoses in patients with obstructive azoospermia. Typically, the testes are of normal size as spermatogenesis is unaffected. The vasa are not palpable, and the epididymal remnants are distended. The caput of the epididymis is always present. Palpation is therefore the key to diagnosis.

It cannot be overstated how easy the diagnosis is with careful attention to the details of the physical examination. Because the seminal vesicles are absent or aplastic, there is no contribution from them to the ejaculate and, consequently, seminal volume is low (<1.0 cc) and the pH is acidic (<7.0). In the azoospermic man with an ejaculate volume of 0.6 cc, a seminal fluid pH of 6.5, and no palpable vasa, the diagnosis is straightforward. The ejaculate consists only of prostatic secretions. Microsurgical epididymal sperm harvesting is employed in conjunction with ICSI to help these couples achieve pregnancy. The epididymal sperm can be used equally well in either the fresh or frozen-thawed state.

**The role of the cystic fibrosis gene**

Cystic fibrosis (CF) represents one of two main genetic etiologies for CBAVD. It is an autosomal recessive disease that occurs in 1 in 1,600 people of northern European descent. The CF gene is located on chromosome 7. The gene product is a transmembrane protein termed CFTR, which regulates the viscosity of epithelial secretions in the respiratory tree and pancreatic ducts by controlling the membrane flow of sodium and chloride ions. In CF, pulmonary secretions are thick and tenacious and set up chronic respiratory infections and airway obstruction. In the pancreatic ductal system, secretions are also quite viscous and, over time, destroy the exocrine function of the pancreas. Pulmonary physical therapy and pancreatic enzyme replacement are the mainstays of treatment for the patient with CF.

The most common mutation of the CF gene, ∆F508, occurs in 60% of CF chromosomes worldwide. Up to 1,000 other mutations have been defined, but most are quite rare. Mutations in both paternal and maternal alleles must occur for a person to express disease. If only one allele is affected, the person is clinically healthy and is termed a “carrier.”

All males with CF will have vasal aplasia and will be infertile as a result. Anguiano and colleagues published the first peer-reviewed investigation demonstrating that males with CBAVD had CF gene mutations as the basis of their reproductive ductal abnormalities.

CF and CBAVD are yet another example of the phenotypic spectrum that is seen in genetic diseases. When both mutations are considered “severe,” the patient will develop clinical CF, and when the mutations completely abolish CFTR function, the patient will be afflicted with the most severe form of CF. However, when the two mutations in combination are “less severe” or “milder,” the pulmonary and pancreatic systems may be unaffected clinically and vasal agenesis may be the only outward manifestation.

All men who present with CBAVD thus require a CF mutation analysis prior to intervention. However, it is not enough to screen only the patient’s partner. In taking a much more global view, the patient with CBAVD is likely to have siblings of reproductive age. He
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may simply be the first in his family to have recognized CF mutations inherited from both his father and mother. The patient needs to know that his siblings are likely to be CF mutation carriers, if they don’t have disease themselves, and that family screening is an integral part of taking care of the whole patient.

Partner screening is critical to the couple to define their risk of transmission of CF or CBAVD to any conceived offspring. It is necessary to test both patient and partner for mutations that occur not only in patients with CF but also in those with CBAVD. One of the most common of these mutations is the so-called 5T allele. Figure 6 shows a Punnett square illustrating the phenotypic possibilities for the offspring when the female partner is a carrier of the ΔF508 mutation and the patient is a compound heterozygote for ΔF508 and R117H. Statistically, 25% of conceived offspring will be affected with CF, and 50% will be carriers of a CF mutation; 25% of male offspring may express CBAVD. Patients with CF mutation-related CBAVD will have normal kidneys.

When one vas is palpable, the semen volume is low, and the seminal fluid pH is acidic, the seminal vesicle on the side of the palpable vas is probably not functional. The vas itself is the seminal vesicle on the side of the palpable vas is probably not functional. The vas itself is

The role of mesonephric duct abnormalities

A second genetic etiology for CBAVD involves abnormal differentiation of the mesonephric ducts. At or around week 7, the mesonephric duct splits into two subdivisions. The first is involved with renal and ureteral development, the second with reproductive ductal development. If a genetic aberration affects mesonephric duct differentiation prior to week 7, bilateral renal and reproductive ductal agenesis will occur, and the baby will die after a few weeks of life.

Patients with CBAVD and unilateral renal agenesis may have a slightly less severe phenotypic form of the same genetic etiology and no CF mutations. Therefore, it’s possible for them to pass it along to their offspring. It is important to counsel these couples about the possibility that any fetus could be afflicted with bilateral renal agenesis.

McCallum et al. reported on a cohort of patients and described a fetus with bilateral renal and vasal agenesis after fetoscopy after termination of the pregnancy. They also described 10 other healthy infants, each with two normal renal units. The genetic basis is unknown as is the transmission pattern. However, real-life data suggest that it is much more likely to have an offspring with normal renal anatomy than it is to have a child with a unilateral or bilateral renal anomaly. This is encouraging news for the couple, but a level 3 ultrasound during early pregnancy is warranted.

On the horizon

There are many other rare conditions resulting in male reproductive compromise whose genetic bases are now being explored and discovered. The most common that we see in practice, however, are the ones discussed in this review. It is imperative to proceed with the appropriate testing prior to intervention as we should not make assumptions on behalf of couples. This applies not only to the azoospermic male but also the patient with severe oligospermia.

REFERENCES