

INDICATIONS FOR GENETIC EVALUATION OF MEN IN A REPRODUCTIVE MEDICINE PROGRAM

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ABSTRACT

The technique of in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) has become widespread for the treatment of severe forms of male factor infertility. Although ICSI has allowed many men previously felt to be poor candidates for IVF the chance for biologic paternity, there have been concerns raised regarding possible transmission of abnormal genes to offspring using this technique. In particular, infertile men with severe defects in sperm production have been shown to have a significantly higher rate of genetic abnormalities than fertile men (1-3). IVF with ICSI clearly bypasses critical natural selection barriers that normally might prevent genetic disease transmission. For this reason, without long-term follow up and evaluation of children born using this technique, there is now a greater need for genetic evaluation in some couples undergoing IVF with ICSI. The reasons for evaluating possible genetic abnormalities include the determination of a cause of the infertility, and the risk of genetic propagation of disease. We now have a clearer understanding of how known, established genetic defects such as cystic fibrosis transmembrane regulator (CFTR) gene mutations and Klinefelter syndrome, for example, affect infertile couples and their offspring. The last several years, however, have led to several advances in genetic detection such as the delineation of Y chromosome abnormalities and the identification of increasingly common chromosome structural abnormalities in men with severe infertility. For men who remain currently untreatable even with ICSI, there may soon be the potential for fertilization with more primitive cells in the spermatogenic pathway such as round spermatids. The genetic implications of this new genetic vector remain unknown. In addition, we may soon be capable of repopulating testes devoid of germ cells with stem cells capable of undergoing complete spermatogenesis. This review examines recent genetic advances in the area of male infertility and summarizes current indications and testing available for genetic evaluation in infertile men.

Key words: male infertility; reproduction; genetics; azoospermia; microdeletions

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INTRODUCTION

A male factor is currently diagnosed in nearly 50% of infertile couples. Despite this, roughly half of these men continue to have an unknown or idiopathic cause for their subfertility. Recently, however, genetic advances have made it possible to classify many disorders previously considered idiopathic. This has led to the understanding that men with severe forms of infertility may harbor genetic defects as the primary cause of their infertility. Prior to ICSI, with

natural intercourse, intrauterine insemination (IUI) or even standard IVF, only the best sperm were capable of penetrating the egg, thus leading to fertilization. It has been known for some time that poor sperm morphology, for example, leads to significantly reduced success with standard IVF (4). With ICSI, these types of natural selection barriers are lost. Indeed, the "selection" of sperm for ICSI involves the mere microscopic appearance of an intact sperm chosen by the ICSI technician. This type of selection may be concerning in that as many as 19% of sperm from nor-

mal men have chromosome structural abnormalities when tested with the sensitive technique of fluorescent in situ hybridization (FISH) (5). Similarly, men with severe oligospermia (< 10 million sperm/ml), but who have plentiful sperm to undergo ICSI, are at risk for harboring Y-chromosome deletions that have been shown to be passed to male offspring (6). Men born with congenital bilateral absence of the vas deferens (CBAVD) often have cystic fibrosis gene mutations despite exhibiting no other sequelae of the disease. As more primitive spermatogenic cells become feasible for use with ICSI, there will no doubt be further concerns for the possibility of propagating genetic defects. Thus, genetic testing and counseling is becoming increasingly important in men with severe infertility.

One diagnostic tool that may become more useful for severely infertile men undergoing ICSI is preimplantation genetic diagnosis (PGD). PGD allows for genetic evaluation of embryos derived from ICSI procedures prior to their reimplantation. This technique is accomplished through genetic evaluation of a single cell taken as a microscopic biopsy from an early 8-cell embryo. Single cell analysis can then be done either with standard karyotyping, FISH, or the polymerase chain reaction (PCR). PGD can be used to detect chromosome numerical and structural disorders, Y-chromosome deletions and CF gene mutations. Other disease states such as XYY syndrome, Tay-Sachs disease, fragile X syndrome and Duchenne's muscular dystrophy can also be evaluated (7). As our ability to detect genetic abnormalities improves using molecular techniques, this will be an increasingly common procedure done in conjunction with ICSI.

Because the technology of IVF continues to rapidly evolve, it is critical that clinicians offer and understand genetic testing available to the couples whom they are treating. Despite known genetic risks, there remains no consensus from organizations such as the American Society of Reproductive Medicine, American College of Obstetrics and Gynecology and the American Urologic Association regarding specific indications for genetic testing in couples undergoing ICSI. It is generally accepted, however, that men with either azoospermia or severe oligospermia (< 5 mil-

lion sperm/ml) are at the greatest risk of harboring genetic defects and thus should undergo testing. In addition, those in whom a suspicion for genetic disease exists based on family history, phenotype, or past IVF outcomes, should also undergo tailored genetic testing.

The goal of this review is to examine our clinical approach to the diagnosis of genetic abnormalities in infertile men. The focus will be on specific genetic conditions that can be diagnosed during the infertility evaluation. For simplicity, genetic disorders will be categorized as classic Mendelian, chromosomal numerical or chromosomal structural disorders. Subsequently, newer methods of genetic testing including preimplantation genetic diagnosis (PGD) will be discussed. Finally, specific indications for genetic testing based on the patient's presenting characteristics will be presented.

MENDELIAN DISORDERS

Mendelian disorders (Table-1) are caused by a mutation at a single genetic locus. These defects can occur de novo or can be inherited through autosomal dominant, autosomal recessive or X-linked patterns (3). Mendelian disorders commonly observed in infertile men are described below.

Cystic Fibrosis Transmembrane Gene Mutations (1:2500)

Cystic Fibrosis (CF) is the most common autosomal recessive disease in caucasians with an incidence of 1:2500 births and a carrier frequency of 1:20 (3). The cystic fibrosis transmembrane regulator gene (CFTR; 7q31.2) was cloned in 1989 and encodes a cyclic adenosine monophosphate-regulated chloride channel found in many secretory epithelia (8). To date, over 800 mutations have been identified in the CFTR gene (9).

Clinical features of CF include chronic pulmonary infection, exocrine pancreatic insufficiency, neonatal meconium ileus and male infertility. Over 95% of men have abnormalities in Wolffian Duct derived structures manifested most commonly as congenital bilateral absence of the vas deferens (CBAVD) (1,2). Anatomically, the body and tail of

Table 1 - Mendelian gene disorders in infertile men.

Disorder	Incidence	Clinical Features	Genetics	Diagnosis
Kallman Syndrome	1:30,000	Anosmia, cleft palate, small testes, infertility	Variable; X-linked recessive (Kalig-1 gene defect)	Clinical
Androgen Insensitivity Syndromes	1:60,000	Variable from virilized infertile male to normal appearing female genitalia with cryptorchidism	X-linked; defect androgen receptor Zq11-12	Clinical or DNA studies
Immotile Cilia Syndrome	1:30,000	Immotile sperm, rhinitis, bronchiectasis, nasal polyposis	Autosomal recessive	Clinical, exam of cilia or flagella by electron microscopy
Cystic Fibrosis	1:2,500	Respiratory infections, pancreatic insufficiency, Wolffian duct anomalies	Autosomal recessive; defect in CFTR gene chromosome 7q31.1	DNA mutation analysis
CBAVD	1:1000	Healthy except Wolffian duct atresia	Compound heterozygosity of CFTR gene	DNA mutation analysis
ADKD	1:400-1:1,1000	Multiple cysts in kidneys, pancreas, epididymis, seminal vesicle; berry aneurysm; renal failure	Autosomal dominant; 3 genes implicated: PKD 1, 2, 3	Clinical syndrome, DNA analysis available
Myotonic Dystrophy	1:10,000	Myotonia, muscle wasting, testis atrophy	Autosomal dominant; expanded CTG DNA repeats in 19q13.3	Clinical syndrome; DNA analysis available

CBAVD = congenital bilateral absence of the vas deferens; CFTR = cystic fibrosis transmembrane conductance regulator gene; ADPK = autosomal dominant polycystic kidney disease.

the epididymis, vas deferens, seminal vesicles and ejaculatory ducts are affected, but the testicular efferent ducts and the caput epididymis are invariably present due to the non-Wolffian derived nature of these structures. Spermatogenesis is usually but not necessarily normal in affected men (10). Because of the potentially fatal and incurable nature of the

disease, both men with CBAVD and their partners should undergo CF testing.

About 2% of infertile men not suffering from cystic fibrosis are found to have CBAVD (11). The CFTR gene contains 27 exons and is 250 base pairs in length. A three base pair deletion in exon 10 (delta F508) accounts for 70% of the mutations found in

the caucasian population. Of note is that considerable variation exists amongst different racial groups (3). Fifty to 80% of men with CBAVD and 43% with congenital unilateral absence of the vas (CUAVD) have detectable CFTR gene mutations (12,13). Interestingly, Mickle et al. showed that those patients with CUAVD and CFTR gene mutations often had a non-iatrogenic contralateral vasal occlusion located superior to the scrotum (14). CFTR gene mutations have also been found in men with CBAVD but without other manifestations of CF (15). It is now understood that a majority of these men are compound heterozygotes for different mutations in each allele of the CFTR gene. A common mutation consists of a DNA variant in a non-coding sequence, the 5T (thymidines) allele in the 3' splicing region of intron 8 (15). Seven or nine thymidines usually occur in this region; a reduction to the 5-thymidine variant decreases the efficiency of splicing of exon 9 and eventually leads to a 10-50% reduction in CFTR mRNA (3). This reduction in mRNA appears to lead to CBAVD without the systemic features of CF. The risk of CFTR gene mutations in patients with nonobstructive azoospermia (NOA) remains unclear. While some studies have shown these mutations to exist in patients with idiopathic testis failure, others have not shown CFTR gene mutations in these patients (16). We continue to recommend that all patients with CBAVD, CUAVD or idiopathic obstruction be screened for CFTR gene mutations.

Generalized Wolffian duct anomalies can also occur in patients with CBAVD. These include partial epididymal aplasia and seminal vesicle aplasia or hypoplasia, which may lead to low ejaculate volume. Secondary findings can include ipsilateral renal agenesis in 11% with CBAVD and in 26% with unilateral vassal absence (13). Imaging confirmation of renal agenesis is imperative in patients with unilateral absence of the vas deferens or in those with CBAVD lacking detectable CFTR gene mutations.

Kallmann Syndrome (1:30000)

Kallmann syndrome, or idiopathic hypogonadotropic hypogonadism, is inherited as a familial disorder in one third of cases. Both X-linked and autosomal inheritance patterns have been described

(1). In the X-linked recessive form, deletions occur in kalig-1 (kallmann-interval 1 gene), a gene responsible for the migration of GnRH neurons to the pre-optic area of the hypothalamus during development (17). As a consequence, there is failure of testicular stimulation by the anterior pituitary and resultant testis failure. In recent animal work, defects in the GnRH receptor itself have been observed that suggest the mechanism for a second, autosomal inheritance pattern (18).

The clinical manifestations of Kallmann syndrome depend on the degree of hypogonadism. Most patients experience a delay in puberty although those with less severe defects may present with a normal appearing phenotype and only subfertility. Other findings include anosmia, cleft palate and small testes. Testicular biopsies can demonstrate a wide range of findings from focal areas of complete spermatogenesis to germ cell aplasia (9). Medical treatment is highly successful and consists of gonadotropin replacement over a 12-18 month period, which induces sperm in the ejaculate in 80% of affected men (19). Often, gonadotropin replacement and eventual stimulation of spermatogenesis can be accomplished with human chorionic gonadotropin alone. In cases where this fails, the addition of human menopausal gonadotropin is useful.

Androgen Receptor Gene Mutations (1:60000)

Over 300 mutations have been found in the androgen receptor, a large steroid receptor gene located on the X chromosome (Xq11-q12) (1). In addition to well-recognized mutations within the gene's 8 exons, mutations in the gene promoter region have also been reported (3). Because many mutations exist, the syndrome is clinically variable and ranges from phenotypic females (complete androgen insensitivity, testicular feminization) to normally virilized but infertile males (20). Depending on the severity of the defect, serum testosterone levels can be low, normal or high. The androgen concentration in each individual depends on the functional integrity of the androgen receptors within the pituitary and hypothalamus.

Recent genetic research on the androgen receptor gene has also led to interesting new clinical

correlation with male infertility. The androgen receptor gene has 8 exons and it is known that a critical region of CAG-nucleotide repeats, usually 15-30 in number, can be found in exon 1 (4). Elongation of this repeat region results in spinal and bulbar muscular atrophy (Kennedy disease), a neurodegenerative disorder that begins around age 30 and consists of muscle cramping and atrophy as well as infertility from testicular atrophy. There is now evidence that subtle abnormalities in this CAG repeat region may also underlie some cases of idiopathic infertility. Yoshida et al. recently detected longer than normal CAG nucleotide repeats in normally virilized men with normal genitalia and idiopathic azoospermia (21). These added CAG nucleotides may be the result of a failure to recognize and repair DNA abnormalities in dividing cells. Whether this represents a more global DNA repair enzyme problem is currently unknown. This does, however, suggest that defects in the androgen receptor may underlie more of male infertility than previously recognized.

Immotile Cilia Syndrome (1:30000)

The hallmark of this heterogeneous disease is impaired or absent ciliary and/or flagellar motility. Clinical manifestations include chronic cough and sinus infection, nasal polyposis, bronchiectasis, and infertility. Kartagener's syndrome, a particular form of this disease (1:60000) consists of situs inversus, bronchiectasis, chronic sinusitis and infertility. Immotile cilia syndromes are characterized by abnormal cell axonemes, both in cilia and sperm tails. The normal axonemal arrangement is 9 outer microtubular doublets with inner and outer dynein arms surrounding 2 central microtubules. In this disease, various defects in either the microtubule and/or dynein arm assembly have been reported (22). It is thought that flagellar function is controlled by the action of over 200 distinct genes (3). Although no single gene to date has been found, the inheritance pattern derived from family pedigrees suggests that transmission is likely to be autosomal recessive.

While infertility is universal in patients with immotile cilia syndromes, ejaculated sperm can be motile and sperm concentrations can be normal or even high. With ICSI, clinical pregnancies and live

births have now been reported from men with this syndrome (22,23). The use of unselected, immotile sperm from such men carries normal fertilization rates of 20 to 40% (24). However, with the use of techniques like hypo-osmotic swelling to select viable, nonmotile sperm, fertilization is possible (25). Because the gene defect is usually recessive, normal offspring are likely in these cases; still, genetic counseling is critical. Interestingly, a novel association between immotile ciliary syndromes and autosomal dominant polycystic kidney (ADPK) disease has recently been observed in Japanese patients: 25% of men with immotile cilia syndromes in this study also had ADPK (26). The pathophysiological relationship between these two disorders is currently unknown.

Autosomal Dominant Polycystic Kidney Disease (ADPK)(1:400-1:1000)

Numerous large cysts of the kidneys, liver, pancreas and spleen characterize this autosomal dominant disorder, and a 10-40% chance of developing berry aneurysms in the brain. Because the syndrome is often asymptomatic until adulthood, affected men may initially present with infertility. Cysts in the epididymis, seminal vesicle or ejaculatory ducts that compress the ductal system and cause obstruction (26) cause infertility. Three separate genetic loci have been associated with ADPK. PKD1 accounts for 85% frequency of the disease and is found on chromosome 16p13.3, PKD2 has been mapped to chromosome 4q, and PKD3 is currently unmapped (3,27). Azoospermic ADPK patients can produce offspring with ICSI using sperm harvested from the epididymis or testis, necessitating appropriate genetic counseling.

CHROMOSOMAL DISORDERS AND MALE INFERTILITY

Chromosomal disorders (Table-2) are defined as the loss, gain or abnormal arrangement of genetic material at the chromosomal level. These disorders can be further divided into numerical and structural abnormalities. Among numerical abnormalities, polyploidy is defined as a chromosome number that is an exact multiple of 23 (excluding 46) (2). Aneuploidy is the gain or loss of one or more chromosomes, such as in

Table 2 - Chromosome numerical and structural disorders found in infertile men.

Disorder	Incidence	Clinical Features	Genetics	Diagnosis
Klinefelter Syndrome	1:500-1:1,000	Tall stature, small firm testes, infertility, mild mental retardation, breast cancer, extragonadal germ cell tumors	90% 47,XXY; 10% one of 30 described mosaics (46,XY/47,XXY)	Karyotype
Y-chromosome Microdeletions	15% Azoospermic and 5% oligospermic men	Normal	Unknown; DAZ gene defects can be passed from infertile fathers to male offspring through ICSI	Peripheral blood genotyping; possible need for sperm cell genotyping
XYY Syndrome	1:250-1:1,000	Tall stature; 1-2% aggressive behavior; infertility	47,XYY	Karyotype
Noonan's Syndrome	1:1,000-1:2,500	Short stature, webbed neck, low set ears, hypertelorism, cardiac anomalies, cryptorchidism	Autosomal dominant, 46,XY	Clinical
Mixed Gonadal Dysgenesis		Testis (cryptorchid) on one side with streak gonad on other side	45,X/46,XY	Karyotype, clinical
46 XX male	1:20,000	Male with small, firm testes	46,XX, SRY gene translocation	Karyotype

Klinefelter syndrome (47,XXY) or Down syndrome (trisomy 21). These defects can occur in all cells or only in some cells, a condition termed "mosaicism". Structural chromosome disorders can occur in single or multiple chromosomes and are becoming more frequently recognized as contributing factors to infertility. Examples of defects that occur in a single chromosome are deletions, duplications and inversions. Translocations, either balanced or unbalanced, occur between 2 chromosomes.

Chromosomal disorders are found much more frequently in the infertile men than in fertile individuals (28). These chromosome disorders currently can be detected in 15% of azoospermic and 5% of oligospermic men, and now represent one of the most common genetic defects in infertile men (29,30). Recognition of these defects will continue to improve as our ability to detect them improves. With ICSI, infertile patients with these disorders that were previously considered untreatable can now be offered paternity

(28). As such, it is important that these couples undergo genetic testing.

Klinefelter Syndrome (1:500-1:1000)

Klinefelter Syndrome (47,XXY) is 45 times more common in men seeking treatment for infertility than in the general male population and is the most common numerical chromosome anomaly seen in male infertility (28). About 90% of men have the classic 47,XXY genotype; the remainders have any number of 30 recognized mosaic patterns (1). Clinically, fewer than 5% of patients present before puberty. Most men present in adulthood with tall stature, gynecomastia (25%) and infertility. Affected men are also predisposed to diabetes mellitus, varicose veins, chronic bronchitis, extragonadal germ cell tumors and breast tumors. Among infertile men, the classic phenotype is a patient with small, firm testes, elevated serum FSH, and low serum testosterone and elevated estradiol levels. Patients are commonly azoospermic and testis biopsy often reveals hyalinization of the seminiferous tubules with Leydig cell hyperplasia. However, there may be focal areas of spermatogenesis (9). The mosaic variant is less severe and can present with normal testicular size, complete spermatogenesis and the presence of ejaculated sperm.

Pregnancies in infertile men with Klinefelter syndrome have now been reported using ICSI (31). For this reason, there has been recent interest in determining germ cell ploidy (number of sex chromosomes) in sperm obtained from these patients to assess whether sperm are normal haploid (X or Y chromosome present) or hyperhaploid (XX, XY etc. present). In one study, the risk of non-disjunction and hyperhaploid sperm production as determined by 3-color FISH was estimated to be 3.9% of all sperm (32). With the use of preimplantation genetic diagnosis (PGD), it is now possible to detect abnormal aneuploid embryos and avoid their reimplantation (33).

47,XXY Syndrome (1:250 - 1:1000)

The genetic cause of this syndrome is paternal non-disjunction during meiosis that results in YY sperm (3). These patients exhibit tall stature, and 1-2% show aggressive behavioral characteristics. Most are azoospermic or severely oligospermic and testis

biopsy findings range from Sertoli cell-only to maturation arrest patterns (9). Unlike Klinefelter syndrome, serum testosterone levels are normal.

Noonan's Syndrome (1:1000-1:2500)

Features of this clinical syndrome are similar to Turner syndrome in females and include short stature, webbed neck, hypertelorism, low set ears, cubitus valgus, ptosis, and cardiovascular anomalies. While an X-linked dominant pattern of inheritance was originally suspected, it is now known that at least one gene defect is inherited in an autosomal dominant manner on chromosome 12 (1). Patients typically have hypogonadism with low testosterone and high pituitary gonadotropins. Cryptorchidism and testicular atrophy are commonly observed in this condition.

Mixed Gonadal Dysgenesis

Patients with mixed gonadal dysgenesis usually have a mosaic 45,XO/46,XY genotype and anatomically have a testis on one side and a streak gonad on the other (1). Often, the normally formed testis is intraabdominal and devoid of viable germ cells. The streak gonad is at risk of developing gonadoblastoma or seminoma and should be surgically removed. There are varying degrees of ambiguity of the external genitalia.

Translocations and Inversions

An increasing number of men with previously diagnosed idiopathic infertility are found to harbor chromosomal translocations and inversions as our ability to detect these defects improves. In men with very low sperm counts, subtotal structural abnormalities can now be observed in 2 to 4% despite a lack of detectable aneuploidy (30). In these men, the rate of balanced translocations and gene inversions is 8 times that of normal men (30). Roughly half of these defects are Robertsonian translocations between chromosome 13 and 14. Robertsonian translocations occur when the short arm of 2 acrocentric chromosomes (chromosomes with very little genetic material on the short arm) fuses. This rearrangement gives rise to a single chromosome containing the long arms of the two chromosomes while the short

arms are lost. The translocation is considered balanced despite the loss of one chromosome due to the paucity of genetic information present on the lost short arm. These patients typically have severe spermatogenic defects but can produce viable embryos with ICSI (28). However, unbalanced translocations can occur in the offspring. Although male infertility due to translocations is potentially treatable with preimplantation genetic diagnosis, few viable pregnancies have been achieved to date.

Y chromosome Microdeletions

Tiepolo & Zuffardi were first to show that the Y chromosome (Yq11) (Figure-1), might contain an azoospermia factor (AZF) (34). Reijo et al., almost 20 years later, discovered specific microdeletions in the Y chromosome in azoospermic men (Figure-2) (35). At that time, one candidate gene region for AZF was thought to be RBM (RNA Recognition Motif); however, these authors demonstrated that a separate region of Yq was deleted in 13% of azoospermic men

Y Chromosome

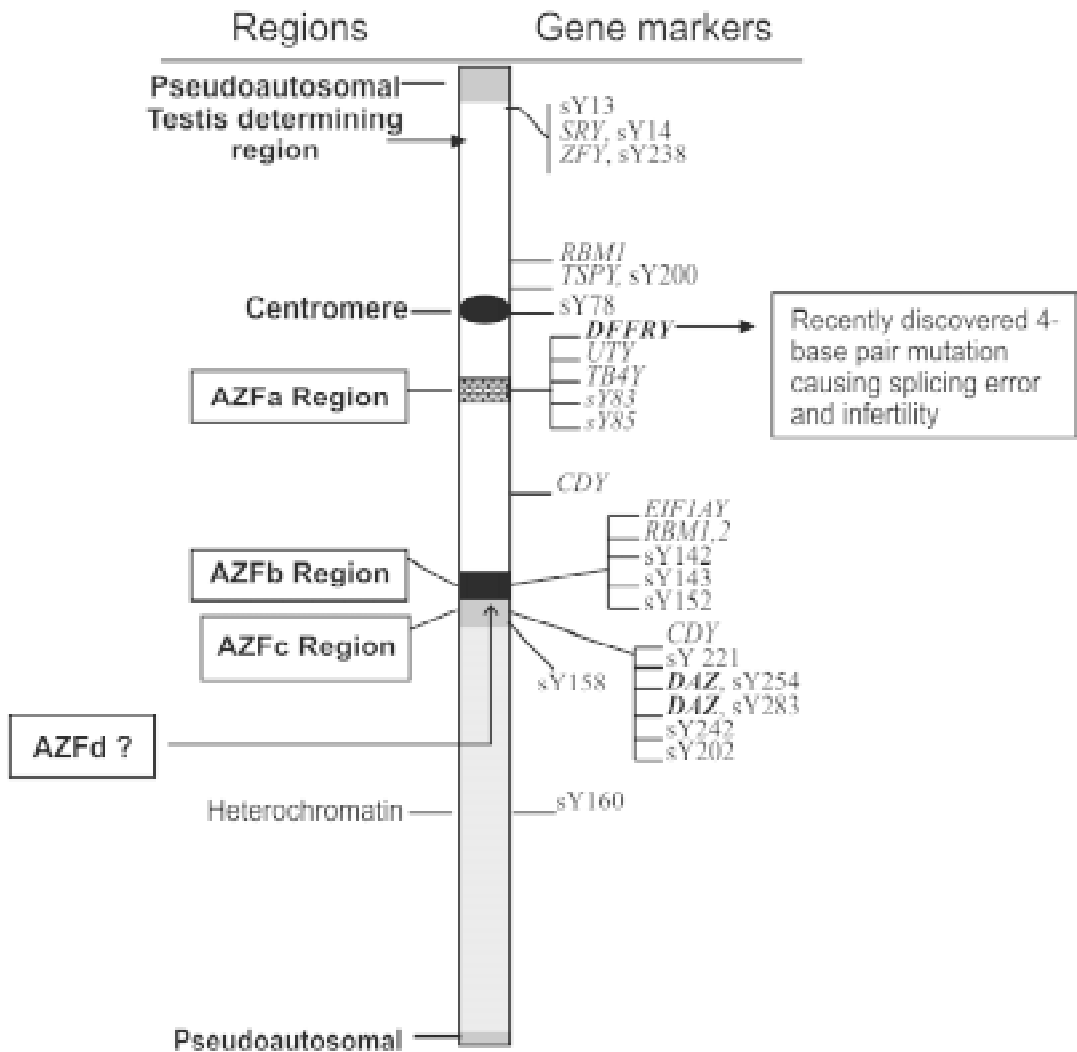


Figure 1 - Gene regions and markers currently known on the Y-chromosome.

and termed this gene DAZ (Deleted in Azoospermia) (35). Subsequently, Vogt et al. described deletions in 3 distinct regions of the Y chromosome, which they termed Azoospermia Factor regions a, b and c (AZFa, b, c) (36). The DAZ gene cluster is found in AZFc (Figure-2). Kent-First et al. have recently suggested the presence of a fourth AZF region (AZFd) residing between AZFb and AZFc that may be associated with mild impairment of spermatogenesis and abnormal sperm morphology (37). Importantly, it is now clear that similar Yq microdeletions also occur in 4 to 8% of severely oligospermic men (38). Taken together, these deletions represent the most common molecularly defined cause of male infertility in humans (39).

Reproductive center to center variation in detection rates of Y chromosome microdeletions is large and is most likely due more to non-standardization of detection techniques than natural differences in populations (40). Another complicating feature of Y chromosome analysis is that some men may harbor DAZ deletions only in germ line tissue (mosaics) and not in somatic cells (6). Thus, the current technique of testing peripheral blood with standard PCR techniques may not be truly reflective of an individual's Y chromosome status in germ-line tissue. The actual status of a patient's Y chromosome for infertility purposes may require analysis of individual sperm cells to define the incidence of this possible mosaicism.

The relationship between AZFa, b and c deletions and the histology of the testis in infertile men remains undefined. In general, variable testis histology can accompany any AZF deletion and ICSI may be potentially successful in all groups (39,41). However, it has been shown conclusively that genetic deletions in the DAZ region can be passed to the male offspring of affected fathers through ICSI (42). Thus, 1 in 10,000 live births could be affected by DAZ deletions if one assumes 1 in 1000 men is azoospermic (2).

The DAZ gene encodes an RNA-binding protein thought to be critical for spermatogenesis (35). During evolution, the DAZ gene complex was most likely transposed to the Y chromosome since many other organisms have autosomal homologues to the DAZ gene (so called DAZ-Like (DAZL)). Recently, an autosomal homologue to human DAZ has been

found on chromosome 3 but its function is unknown. The DAZL genes in other species have strong functional relationships to fertility. Homozygous DAZ-deleted mice exhibit sterility whereas heterozygous mice are only subfertile. Disruption of the DAZL homologue *boule* in flies results in maturation arrest and sterility. Such variation in the degree of infertility with deletion status suggests that the copy number of normal DAZ/DAZL may influence the eventual fate of male germ cells (38). In humans, the DAZ protein product is present in high concentration in premeiotic germ cells (43), corroborating findings from other studies that localize DAZ mRNA to similar cell populations in the mouse (44).

Meiosis and DNA Repair

Maturation arrest is commonly observed in testis biopsies from infertile men with nonobstructive azoospermia. The morphological point of arrest (primary spermatocyte) is suggestive of a defect in meiosis, since secondary spermatocytes have already undergone crossing-over of DNA, the critical step whereby DNA is exchanged between the maternal and paternal alleles. Recently, many genes required to complete meiosis in other organisms have been identified (45). Some of these genes appear to tightly regulate the recombination of genetic material and the repair of obligate DNA breakages that are essential for recombination to occur during meiosis. In organisms such as yeast and mice, mutations in such genes required for DNA repair lead invariably to infertility characterized by meiotic arrest (possible equivalent to human maturation arrest) (46).

There is literature to suggest that defects in DNA repair may also underlie human infertility. Pearson et al. observed a reduced ability to repair induced DNA damage in lymphocytes from an azoospermic man with arrested germ cell growth (47). Solari et al. recently found abnormal meiotic synaptonemal complexes in testis biopsies from infertile men (48). These observations suggest that human maturation arrest may indeed be genetic and may be linked to mutations in genes required for DNA repair.

Recently, using microsatellite DNA analysis to evaluate DNA repair fidelity, we have found that the ability to repair DNA mismatches is altered in some

infertile patients with maturation arrest (49,50). In these men, DNA repair defects occurred only in the germ cells (dividing by meiosis) and not in somatic

cells (dividing by mitosis) where DNA recombination does not occur (50). Some individuals with incomplete maturation arrest (sperm present focally)

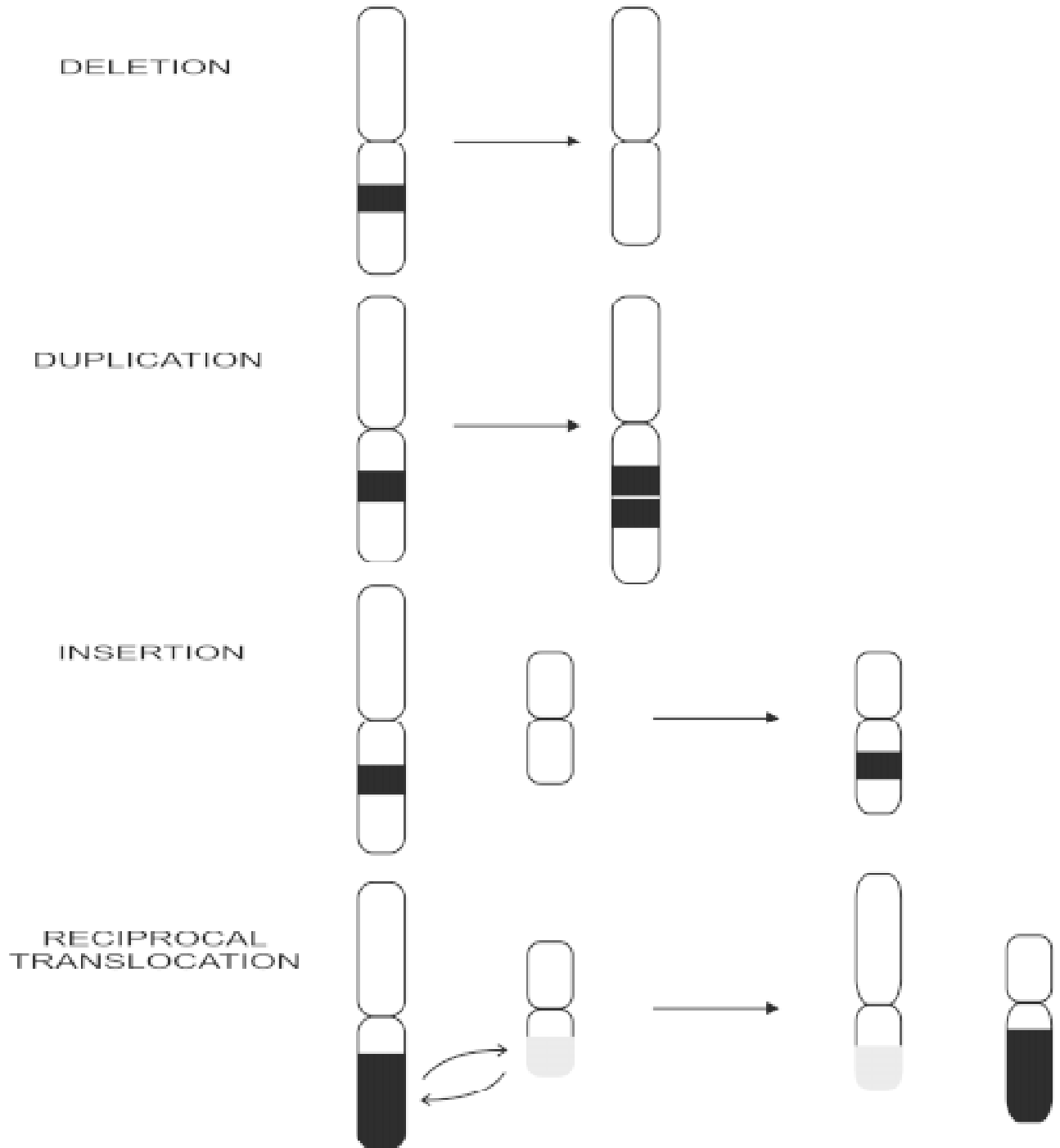


Figure 2 - Structural chromosome abnormalities including deletions, gene duplications, insertions and translocations. The lower part depicts a reciprocal, balanced translocation.

also were found to harbor similar defects in DNA repair as men with complete arrest. Some of these men will have enough sperm present for ICSI and thus could pass DNA repair defects to their offspring. At this time, however, the relationship of defective DNA repair in germ cells and the subsequent health of children conceived by ICSI is not known.

NEW DIRECTIONS IN GENETICS

Spermatid Injection

Several reports have shown that fertilization and cleavage of embryos can be achieved with both elongated and round spermatids, potentially offering paternity to men with late maturation arrest (51,52). Fertilization rates approaching that seen with fully mature sperm (50 to 71%) have been observed with elongated spermatids (ELSI) and several live births have been reported (51). However, the fertilization rates with round spermatid injection (ROSI) are still quite disappointing (20 to 25%) (51,53). Despite the poor efficiency of the technique, there have been reports of live births from ROSI (52). Whether these births occurred with the use of round spermatids or elongated spermatids remains controversial (54). The question of whether round spermatids have undergone all of the critical genetic maturation processes necessary for normal development remains unanswered.

Germ Cell Transplantation

The concept of transplanting early spermatogenic cells offers the hope of germ cell repopulation in sterile testicles and forms a basis for research in totipotent stem cells (stem cell renewal) (55). Recently, studies have shown that rat spermatogonial cells that are transplanted into the seminiferous tubules of immunodeficient mice can generate rat spermatogenesis (56). In addition, it may now be possible to cryopreserve spermatogonial stem cells (56) and propagate them in culture to encourage their development and maturation into later spermatogenic forms (57). Clearly, this would be useful in men undergoing chemotherapy or radiation therapy for malignancies. Research in this field in the next decade has tremendous promise to enrich the field of male infertility from both scientific and clinical viewpoints.

Preimplantation Genetic Diagnosis (PGD)

The importance of early detection of genetic disorders in the offspring of couples treated with ICSI is critical. The first embryo biopsy was performed in 1990 and used the polymerase chain reaction (PCR) to detect Y-chromosome bearing sperm in order to avoid implantation of embryos with an X-linked disorder (58). As of 1998, normal embryos have been identified and replaced with the help of PGD from patients with genetic diseases ranging from Mendelian gene disorders to those with numerical or structural chromosomal defects (33). Most couples undergo PGD for one of 3 reasons: a)- genetic risk with previous spontaneous abortion, b)- genetic risk with objection to therapeutic abortion, and c)- genetic risk with subfertility (7). Embryo biopsy success rate (97%) and the accuracy of PGD in diagnosing specific abnormalities have been very high in general, with only one known misdiagnosis as of 1997 (33). However, because of the complexity and invasive nature of the technique, the overall pregnancy rate for PGD embryos is lower than those observed with standard ICSI (17.6% vs. 25-33%).

PGD has been used to diagnose many of the genetic conditions described in this chapter. For CF gene mutations, Handyside et al reported the first use of PGD to detect the delta F508 mutation (59). It has now been shown that several other CF gene mutations can be rapidly evaluated from a single cell PGD biopsy (60). Although technically feasible, the use of PGD for Y-chromosome deletions has not been described. This may be due to the lack of a noticeable disease state in the affected offspring. Perhaps most importantly, PGD has been used to detect chromosomal aneuploidies using multiprobe FISH (61). This is most useful in patients with mosaic Klinefelter syndrome at risk of propagating sex chromosome aneuploidy or those with advanced maternal age at risk of Down syndrome. Finally, PGD has been used to detect reciprocal translocations in chromosomes 5 and 8 (64) as well as Robertsonian translocations (63). The detection of Robertsonian translocations may be important because it has recently been shown that significant pregnancy rates are achieved despite the presence of this defect (64).

CONCLUSIONS

As our ability to bypass the detrimental effects of genetic defects on fertility improves, it is imperative that physicians treating infertile couples understand and continue to examine genetic causes of infertility. There have been tremendous advances in our understanding of some diseases and their relationship to infertility such as that for CFTR gene mutations and CBAVD. We now appear similarly close to elucidating molecular steps in sperm production with our evolving understanding of genes on the Y chromosome and genes involved with meiosis. A greater mechanistic understanding of normal spermatogenesis will not only help explain the cause of infertility in many men, but also the risk these men face as ART techniques become increasingly more widespread. With genetic counseling, family genetic analysis, and evolving PGD technology, many of the genetic risks that severely infertile men bring to ICSI can be potentially avoided in the future.

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