

State of the Art for Genetic Testing of Infertile Men

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Intracytoplasmic sperm injection (ICSI) now provides fertility in many cases of severe idiopathic spermatogenic failure and obstructive azoospermia. Genetic causes must be sought by systematic evaluation of infertile men and affected couples informed about the implications of such diagnoses for assisted reproductive technology outcome and their potential offspring. This review discusses established and emerging genetic disorders related to fertility practice. Chromosomal anomalies are found in about 7% men with idiopathic spermatogenic failure, predominantly numerical/structural in azoospermic men and translocations/inversions in oligospermic men. Routine karyotyping of men with sperm densities less than 10 million/ml, even in the absence of other clinical presentations, is recommended because infertility is associated with higher rates of aneuploidy in ejaculated or testicular sperm and increased chromosomal defects in ICSI offspring. The long arm of the Y chromosome microdeletions are the most common recognized genetic cause of infertility and are found in about 4% men with sperm densities less than 5 million/ml. Routine testing using strict quality assurance procedures is recommended. Azoospermia factor (AZF)-c deletions, the most common form of the long arm of the Y chromosome microdeletions, are usually associated with low levels of sperm in the ejaculate or in testis biopsies, whereas men with AZFa or AZFb+c deletions usually produce no testicular sperm. When AZF-deleted sperm are available and used for ICSI, fertility defects in male offspring seem inevitable. Bilateral congenital absence of the vas is associated with heterozygosity for cystic fibrosis transmembrane receptor mutations making routine gene screening and genetic counseling of the couple essential. Testing for less common genetic associations/defects linked with different reproductive dysfunction may be applicable to specific patients but have not entered routine practice. (*J Clin Endocrinol Metab* 95: 1013–1024, 2010)

Infertility affects one in eight couples of reproductive age and in half; male factors are the sole or a contributory cause. Primary testicular disorders affecting spermatogenesis account for two thirds of cases; most are unexplained and described in terms of semen parameters and described variously as primary spermatogenic failure or idiopathic infertility (<http://www.endotext.org/male/male7/maleframe7.htm>). This diagnostic ignorance frustrates both the patient and physician because without pathophysiological understanding, specific treatment is unlikely. Assisted reproductive tech-

nologies (ART), such as the isolation and harvesting of testicular sperm for use in intracytoplasmic sperm injection (ICSI), are widely used to circumvent limitations in sperm production or function.

The clinician has a duty to inform the couple, where possible, of the reason for the male's disability and identify treatable disorders, even if they are uncommon (*e.g.* gonadotropin deficiency). Furthermore, coexistent diseases more prevalent in fertile populations (*e.g.* androgen deficiency, testis cancer) must be sought and consideration

ISSN Print 0021-972X ISSN Online 1945-7197

Printed in U.S.A.

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doi: 10.1210/jc.2009-1925 Received September 10, 2009. Accepted December 29, 2009.

First Published Online January 20, 2010

Abbreviations: AR, Androgen receptor; ART, assisted reproductive technologies; AURKC, Aurora kinase C; AZF, azoospermia factor; BICAV, bilateral congenital absence of the vas; CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; CNV, copy number variation; ICSI, intracytoplasmic sperm injection; KS, Klinefelter's syndrome; OA, obstructive azoospermia; TESE, testicular sperm extraction; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling; Yq, long arm of the Y chromosome.

given to the implications of treatment for potential offspring. Genetic causes for both spermatogenic failure and obstructive azoospermia are known but collectively account for a minority. Despite much progress in animal systems (1), the validation of novel genetic assessments has been slow, and only a limited suite of tests are currently considered as essential in the evaluation of the infertile male.

The great progress in understanding the genetic regulation of gonadal development, sex determination, and endocrine regulation of puberty is recognized and testing may be applicable in certain centers and family settings. This review, however, will focus on those most commonly recognized genetic causes of male infertility and foreshadow nascent areas of research that may have a clinical impact in the medium term.

Key investigations in male infertility

The clinical description of male fertility status highlights semen parameters and features the use of terms such as azoospermia (no sperm apparent in semen). However, in the assessment of sperm density, it must be recognized that methods, such as those outlined by the World Health Organization, have a detection limit (2) of around 0.15 million/ml when using a standard counting chamber and the recommended procedure. Consequently sperm density would be better described as undetectable (with a limit) rather than azoospermic (3). This concept helps account for occasional (by chance) detection of sperm in the semen of apparently azoospermic men and the isolation of sperm in testicular biopsy material in others (see below). There is no agreed definition of severe oligospermia, but in this review it is taken as less than 5 million/ml unless otherwise stated. Whereas sperm density is a significant prognostic factor in fertility outcomes across populations, there are limitations in application to individual couples' fertility, which is affected by many parameters, and even severely oligospermic men can achieve natural conceptions (4).

Chromosomal anomalies

It is well recognized that infertile men have an 8- to 10-fold higher prevalence of chromosomal anomalies than fertile men and often exhibit no other phenotypic features (5). Hence, there is a need to routinely karyotype infertile men with severe unexplained spermatogenic defects (6, 7). For example, in a review of 11 studies involving 9766 azoo-oligospermic men, sex and autosomal anomalies were found in 4.2 and 1.5% of men, compared with 0.14 and 0.25%, respectively, in a control (newborn) population (8). The chromosomal anomaly rate was highest in the azoospermic subjects (13.7%), being predominantly numerical (*e.g.* Klinefelter's syndrome) or struc-

tural defects (Fig. 1). Within oligospermic men, a 4.6% prevalence of autosomal translocations and inversions has been reported (9). It should be remembered, however, that not all anomalies impact fertility; for example, 0.37% of sperm donors with normal semen parameters have been reported to have chromosomal translocations (10). Consistent with predictions of difficulties with meiosis, men with translocations tend to have hypogonadism, decreased sperm concentration in their ejaculates, and high serum FSH and/or androgen deficiency (11). By extension, the increased rates of chromosomal anomalies (both paternally inherited and *de novo*) in ICSI children (12) most likely arise due to sperm aneuploidy; however, the absolute rate is small (13).

Chromosomal defects that have been associated with male infertility can be classified as numerical, structural (translocations, inversions), or those affecting the Y chromosome.

Numerical defects

Klinefelter's syndrome (KS), 47, XXY, is the most common chromosomal abnormality. Clinicians should consider KS in all infertile men with azoospermia as nonmosaic KS accounts for 11% of cases, whereas mosaic KS (10% total) accounts for about 0.5% of the severely oligospermic population (9). Most KS men are never diagnosed (14) due to a combination of the low awareness of the condition, the prevailing misconception that all have the classic textbook phenotype (tall, gynecomastia, florid hypogonadism), and the failure of clinicians to do a genital examination during routine health care (15, 16). In reality KS has highly variable clinical features; many appear well virilized at first glance and have a wide range of school and workplace achievement that overlaps the general population. The only invariant finding of nonmosaic KS is that of small testes (2–4 ml). The infertility presentation represents a real opportunity for intervention, not only for fertility through the isolation of testicular sperm and ICSI but also in providing the patient with an explanation for his life experiences and possible androgen replacement, which may offer major benefits for somatic tissues and quality of life (16).

The predominant histological picture in KS is of Sertoli cell only and hyalinized tubules (17), but remarkably, small islands of spermatogenesis permit the isolation of testicular sperm in 40–69% of nonmosaic KS (18–21), allowing the use of ICSI and enabling normal live births (22). Reassuringly, most sperm from KS men have a normal 23X or Y complement. Currently it is uncertain whether these arise from 46,XY spermatogonial lineages (23) or the exclusion of the extra X during meiosis. In a newly diagnosed KS man presenting with infertility, the

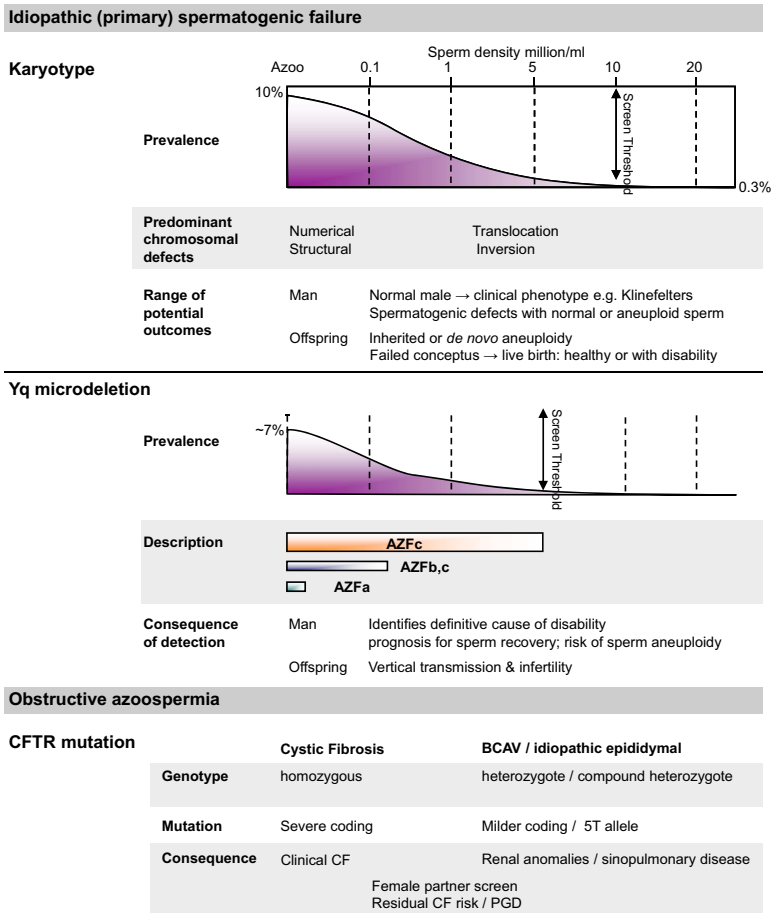


FIG. 1. Essential genetic investigations in male infertility. Karyotype and Yq assessments in idiopathic spermatogenic failure (*top two panels*) are shown with approximate prevalence, types, and consequences to the individual and his offspring. Note the relationship of both to sperm concentration and differing recommended testing thresholds at which abnormalities approach those of the normal population. The approximate prevalence of the AZF microdeletion subtypes are reflected in the size of their boxes. CFTR mutation screening (*lower panel*): in the setting of obstructive azoospermia in CF or congenital absence of the vas (BCAV) or idiopathic epididymal blockage. Note the differing CFTR genotypes but the common need for female partner screening, discussion of residual risk, and consideration of preimplantation genetic diagnosis (PGD).

institution of lifelong androgen replacement should be delayed briefly to allow consideration of testicular sperm extraction (TESE) to ensure adequate gonadotropic drive and potentially improve TESE outcomes (20). If, and for how long (*e.g.* several months), androgens should be withheld from an established KS case to allow a rise in gonadotropins and thus spermatogenesis is unclear. Importantly, rates of aneuploid sperm, although low in absolute terms, are increased in KS men (19, 24) as is the prevalence of aneuploid embryos (both sex and autosomal) (25), making counseling of prospective couples essential.

Individuals with 47,XYY are often fertile but apparently have an increased likelihood of infertility compared with 46,XY men (11) This area has not been examined systematically (7). Interestingly, in the majority of 47,XYY men studied, their sperm have a normal chromosomal

complement (7). Nonetheless, higher rates of sex (26) and autosomal imbalances (27) have been reported within 47,XYY men. The 46,XX-male genotype is rare and usually occurs as a consequence of the translocation of the portion of the short arm of the Y chromosome containing the SRY gene onto one of the Xp arms. This triggers the development of testes, but due to the absence of the long arm of the Y chromosome (Yq), spermatogenesis does not occur (28).

Robertsonian translocations (~0.1% newborns) feature centric fusion of two nonhomologous acrocentric chromosomes (13, 14, 15, 21, and 22) with loss of the short arm material. The most common translocations involve chromosomes 13;14 and 14;21 and are predominantly found in oligospermic (1.5%) rather than azoospermic (0.2%) men who usually have an otherwise normal male phenotype (6, 9). Intriguingly, within some families, fertility is maintained despite the same apparent translocation, *e.g.* t(13;14)(q10;q10), and such translocations are also seen in fertile sperm donors (10). Through interference with spermatocyte chromosomal pairing, arrested germ cell development (azoospermia) may result, but more often oligospermia is seen. Of those sperm that are produced, most have a normal, balanced chromosomal complement, but an unbalanced complement is present in 4–40% of sperm (7, 29) and can have significant implications for offspring, *e.g.* trisomy 21 or 13 or uniparental disomy in relation to chromosomes 14 and 15, resulting in bone and growth disorders such as Prader Willi syndrome.

Reciprocal translocations (~0.1% newborns) are defined as the exchange of material between autosomes or between the X or Y chromosome and an autosome and occur in 0.7% of severely oligo- or azoospermic men (30). The chromosomal complement is normal, and generally the chromosomes and break points are unique to that family. Although most translocation carriers are phenotypically normal, in some cases abnormalities can arise due to the break points interrupting important genes or via position effects, wherein a gene is translocated into a region in which its expression is either up- or down-regulated by enhancer or promoter elements (*e.g.* risk of cancer because the translocation has inactivated a tumor suppressor gene or activated an oncogene). Often carriers have only a spermatogenic phenotype featuring meiotic arrest, but when

sperm are produced, more than 50% are chromosomally unbalanced (7, 31), the percentage varying with the chromosomes involved and the break points.

Chromosomal inversions may involve the centromere (pericentric) or a peripheral component of the chromosome (paracentric). Many are harmless polymorphisms and are detected incidentally, but inversions may have complex outcomes, depending on the chromosome and the site and extent of the inversion. Due to the formation of abnormal loops during chromosomal pairing and disruption of meiosis, duplication and deletion events can occur, resulting in a germ cell arrest or the production of sperm with high rates of aneuploidy and consequent adverse birth outcomes (7, 32, 33).

Recommendation for chromosomal evaluation

Based on prevalence data and the lack of other phenotypic features, routine karyotyping of infertile men with unexplained spermatogenic failure and a sperm concentration less than 10 million/ml is widely recommended before ART (Fig. 1) (34).

We are not aware of cost-effectiveness data underlying this recommendation; such an analysis is complex because many factors bear on this question including: 1) the health burdens of failed ART or abnormal conception and live birth, 2) whether screening may fail to detect and prevent adverse outcomes, and 3) cost issues including the public *vs.* private nature of medicine.

The heterogeneous nature of chromosomal anomalies and their potentially complex reproductive outcomes make it essential that clinical genetics expertise be engaged in modern fertility practice, both for the diagnosis and counseling of couples about natural or ART conceptions. If sperm are available but carry a recognized or unrecognized chromosomal defect, the outcome may be no risk above that of the normal population, a nonviable conceptus, or a significant prospect of live birth with disability. In some cases the genotype may present a zero prospect for sperm recovery, *e.g.* 46,XX male, and as such it may be appropriate to discuss other options such as the use of donor sperm or adoption.

Sperm aneuploidy rates are also increased in some infertile 46,XY individuals (35, 36), arising perhaps as a consequence of defects in meiotic recombination and segregation of the chromatids. Structural studies have revealed abnormal and/or fewer points of recombination in infertile men compared with fertile men, leading to germ cell apoptosis or the generation of sperm with abnormal chromosomal structures (7, 37, 38). Increased rates of sperm aneuploidy have been reported in 46,XY men with severe defects in sperm number, motility and morphology (39, 40), isolated severe morphology defects (41), sper-

matogenic failure and only testicular sperm available (42) and in couples experiencing recurrent failed ART or pregnancy loss.

Evaluation of sperm for aneuploidies, or inversions, in at-risk couples (*i.e.* those with known karyotypic anomalies or severe spermatogenic defects) might help predict ART outcomes and identify suitable candidates for preimplantation genetic diagnosis (7, 36). Nevertheless, such screening is expensive and problematic because there is no consistent single, or even cluster of, chromosomal pattern that permits a convenient panel for reliable detection. In addition, the relatively low absolute risk of abnormal sperm and the lack of clinical trial data showing improved outcomes after screening have meant that sperm aneuploidy/inversion screening has not been widely adopted outside specialized centers. Trial data establishing the threshold level that ought to direct couples to either undertake preimplantation genetic diagnosis or abandon ART would be valuable.

Preimplantation genetic diagnosis using fluorescent *in situ* hybridization can be used in embryo selection in couples with established anomalies or at high risk of sperm aneuploidy and can identify (but not discriminate between) normal and translocated, but balanced, embryos for transfer. Preimplantation genetic diagnosis can detect unbalanced embryos arising from sperm carrying translocations or inversions (43). The sensitivity of fluorescent *in situ* hybridization analysis is, however, limited to the chromosome regions for which the probes are used. Whereas the testing protocol can be individually tailored to ensure a high degree of accuracy, confirmatory chorionic villus sampling or amniocentesis is often recommended. In the future, more detailed screening of infertile men, sperm, and embryos using approaches like comparative genomic hybridization or microarray analysis will likely result in the detection of more subtle structural defects. This may redefine the approach to testing for chromosomal abnormalities and provide a basis for observed sperm aneuploidy in 46,XY men (44, 45).

Yq microdeletions

The identification of the azoospermia factor (AZF) region on the Yq (46), later localized to Yq interval 6 (47, 48), has led to a key test in the evaluation of severe spermatogenic failure. By contrast it is the short arm of the Y chromosome that contains the sex determination gene *SRY*. Yq microdeletions are the most common identifiable genetic cause of spermatogenic failure (49, 50) and in fact involve the loss of 0.8–7.7 megabase regions that encode multiple spermatogenesis genes. The vast majority arise *de novo*, indicating this region is particularly unstable, a fact attributed to repetitive palindromic DNA sequences,

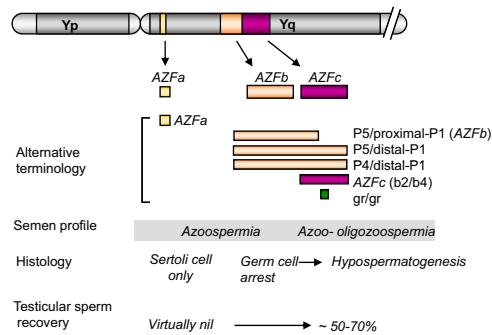


FIG. 2. Yq microdeletions. Deletions along the long arm (Yq) of the Y chromosome have been related to severe spermatogenic failure and described as AZF microdeletions, specifically AZFa, AZFb, and AZFc. Based on definition of the break points, alternative terminology has been suggested (66). A general genotype-phenotype relationship exists in terms of sperm concentration and testicular histology. Sperm are often present in AZFc deletion but rarely with AZFa deletion, even within testicular tissue. The gr/gr subdeletion might be a risk factor for subfertility in some populations. See text for details. Reproduced with permission and modified from S. Repping *et al.*: Nat Genet 35:247–251, 2003 (66). © The Endocrine Society.

termed amplicons, that are clustered along the Yq (51). The high degree of homology between these palindromes promotes intrachromosomal recombination and rearrangements with predictable deletion patterns.

The AZF is comprised of at least three distinct regions; AZFa, which is located on proximal Yq11 (Yq11.21), and AZFb and AZFc, partially overlapping regions, which are located on distal Yq11 (Yq11.23) (52) according to the current model (53) (Fig. 2). Collectively AZFa–c encode gene families for 27 distinct proteins, some of which are Y-specific and others with X or autosomal homologs (54). AZF-encoded proteins appear to have key roles in spermatogenesis, including germ cell cycle regulation and meiosis (55). Despite this knowledge, the molecular basis for impaired sperm production associated with AZF deletions remains unexplained (56). As yet the only AZF gene reported to be deleted is *USP9Y* (located in the AZFa region) that is associated with a heterogeneous phenotype (from azoospermia to normozoospermia), indicating that this gene is most likely a fine-tuner rather than one essential for spermatogenesis. The transmission of *USP9Y* deletions in two families indicate that the protein is not required for sperm-egg interaction (49, 57).

Yq microdeletions are more frequent in azoo- than severely oligospermic (<5 million/ml) men with the prevalence skewed to 0.1–1 million/ml range (Fig. 1). Widely variable estimates reflect patient selection (*e.g.* inclusion of nonidiopathic spermatogenic failure leading to underestimates) but more importantly to methodological deficiencies and false-positive detection, leading to overestimates.

The PCR-based detection methods rely on sequence-tagged sites specific to each AZF region. Multiplex PCR techniques must be optimized to ensure specificity due to

the extensive repetitive elements on Yq. The selection of sequence-tagged site markers is crucial, and specific guidelines for testing must be followed to ensure accurate results (50, 58).

The site and extent of Yq microdeletions affect the infertility phenotype (Fig. 2). Sixty percent of deletions involve only the AZFc region, and in these men, a third have severe oligozoospermia, and in the majority who are azoospermic, more than half have sperm recoverable by TESE (59). Larger deletions involving AZFa and/or b, as well as AZFb+c, are often associated with azoospermia and a poor outlook for sperm recovery by TESE (59–61). In agreement, the histological profile of men with AZFa deletions is usually that of Sertoli cell-only syndrome. Those with AZFb deletions tend to have germ cell arrest at the primary spermatocyte stage; however, patterns ranging from Sertoli cell-only syndrome to severe hypospermatogenesis (*i.e.* presence of some mature spermatids) have been reported (52).

Routine Yq microdeletion testing before ART will inform affected couples of the cause of their infertility and the inevitability of vertical transmission (and likely infertility) in male offspring (62). Interestingly, in Australia AZF-deleted couples rarely seek sex selection via preimplantation genetic diagnosis to prevent transmission of the Yq microdeletion and thus infertility in male offspring. AZFc-deleted sperm provide standard fertilization and pregnancy outcomes (63, 64). A possible decline in spermatogenesis over time in AZFc-deleted men (65) make sperm cryopreservation advisable for future fertility and follow-up of their ICSI-conceived sons in early adulthood is advisable.

In addition to the very large deletions of the Yq described above, several smaller subdeletions exist, the most prevalent of which is termed gr/gr. This deletion removes a 1.6-Mb region within the AZFc region and in doing so removes half of the normal AZFc region gene content (66). The clinical significance of gr/gr deletions remains controversial. Recently two large reports on men of white European descent have provided clear evidence for a significant effect of gr/gr deletion on sperm concentration in the ejaculate (67, 68); the metaanalysis showed that the gr/gr deletion was significantly more prevalent in azoo-/oligozoospermic men than in normal men (odds ratio 2.4) (68). Although a recent multicenter study was unable to provide evidence for a role of Y chromosome racial origin (haplogroup) on the phenotypic variation within European carriers (69), it is interesting to note that in an Australian (largely Anglo-Celtic) population, the presence of gr/gr deletions showed little relationship to sperm concentration, unlike AZFc deletions, but was strongly correlated with male infertility (70). The inclusion of gr/gr test-

ing depends on the data for the patients' racial origin (and therefore likely the whole clinic population), and even then, in counseling couples, gr/gr deletions must be viewed as a risk factor rather than a definitive cause for impaired sperm-production/sub-fertility.

Cystic fibrosis (CF) gene screening

The CF transmembrane conductance regulator (*CFTR*) gene (7q31.2) encodes an epithelial chloride channel for which more than 1200 different mutations are known. CF is a serious autosomal recessive condition with birth incidence of about 1:2500 and a cumulative carrier frequency of one in 25. Preconceptual detection of carrier status allows preventative strategies to be used. Almost all CF males have absent vasa. Interestingly, bilateral congenital absence of the vas (BCAV), in isolation, is a frequent cause of obstructive azoospermia (OA) in apparently healthy men (71). Of these men, 50–82% are heterozygous or carry compound heterozygous *CFTR* mutations (72–75). BCAV can be viewed as the mildest phenotype within the *CFTR* gene mutation spectrum (76). Consistent with this concept are reports of increased rate of abnormal sweat tests and mild sinopulmonary disease in *CFTR*-positive BCAV men (77).

In CF/BCAV, the Wolffian duct derivatives (seminal vesicles, ejaculatory ducts, vasa, epididymal body/tail) appear to atrophy during fetal life (78), giving the classic presentation of OA with normal testis volume; thin/absent scrotal vasa; and a low volume, low fructose, acidic ejaculate. The clinical phenotype includes structural variants, *e.g.* a fibrous cord-like vas may be palpable (71, 79), only the seminal vesicles and proximal vas may be missing, or asymmetry may be apparent. Unilateral congenital absence of the vas with otherwise unexplained obstruction (80, 81) or idiopathic epididymal obstruction similarly has an increased risk of *CFTR* mutation (73, 82). Consequently, *CFTR* screening is indicated whenever suggestive physical finding and/or unexplained OA is present. Finally, renal tract anomalies (hypoplasia/agenesis) affect about 10% of BCAV patients, indicating the need for renal tract evaluation (83–85). Spermatogenesis is usually normal (86) and sperm can usually be recovered from the epididymides or testes for ICSI wherein normal fertilization and embryonic development have been reported (75).

As indicated, BCAV men are frequently heterozygotes for severe coding region mutations (*e.g.* δ -F508) or carry compound heterozygous mutations including milder coding mutations (*e.g.* R117H) and a disease-associated polymorphism (5T, 7T, or 9T). The latter actually occurs within the intronic region of exon 8, and the shorter 5T allele is associated with higher rates of exon 9 deletion and consequently reduced levels of functional *CFTR* protein (87, 88). The types of BCAV/CF causing mutations vary

among ethnic groups, making tailored BCAV screening panels desirable (72). Routine screening is performed for a few dozen prevalent CF-associated mutations but more in-depth screening can double mutation detection rates (73). About 25% of BCAV cases have no detectable *CFTR* mutations (72, 73, 79). However, given the limits of current testing, a negative result does not exclude an unknown mutation and genetic counseling is strongly encouraged.

When a mutation is apparent in the male, *CFTR* screening of the female partner is essential, but even then a negative result leaves a small residual risk of a CF affected offspring. Where *CFTR* mutations are found in both partners, preimplantation genetic diagnosis may identify unaffected or heterozygotic embryos; however, this can be technically challenging if the partners share a mutation and require linkage studies to identify the unaffected allele.

Genetic lesions with established or emerging roles in infertility

Sperm DNA integrity testing

During spermiogenesis the exchange of histones for protamines (protamination) results in a unique, tightly packed DNA structure that is usually less susceptible to damage than somatic DNA. As a trade-off, however, elongated spermatids/sperm have a very limited capacity for repair when damage does occur. When protamination is deficient, such as often occurs in infertile men, sperm remain vulnerable to damage but unable to repair it (89). It has been hypothesized that defective spermatogenesis is associated with an intrinsic vulnerability to stresses, including oxidative DNA damage from reactive oxygen species generated by aberrant sperm mitochondrial metabolism or genital tract leukocytes (90, 91). Indeed, there is an inverse relationship between DNA damage and semen parameters, such as vitality and sperm concentration (92). Whereas oocyte-directed repair of damaged sperm DNA may partially repair the DNA damage, more extensive damage is hypothesized to result in the introduction of mutations and has been associated with poor fertilization or embryo development rates and recurrent miscarriage (93–97).

Methods for assessing sperm DNA integrity include direct measurement of existing DNA breaks [comet or terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assays] or the DNA adduct 8-hydroxydeoxyguanosine as a marker for oxidative DNA damage or the susceptibility to damage, such as mild acid exposure in the sperm chromatin structure assay, which assesses the incorporation of acridine orange into double- and single-stranded DNA and fluorescence flow cytometry. The outputs of sperm

chromatic structure assay, TUNEL, and comet assays are highly correlated (98), as are TUNEL and 8-hydroxydeoxyguanosine (99), suggesting that they measure a common pathological end point.

The clinical utility of sperm DNA testing remains controversial. The American Society for Reproductive Medicine Practice Committee did not find an association with reproductive outcome in either normal or ART couples (100). A metaanalysis of 13 studies involving 2161 *in vitro* fertilization/ICSI treatments revealed a small but significantly increased risk of failed pregnancy associated with high levels of DNA damage but concluded that testing was not clinically useful in discriminating couples who would conceive (101). A subgroup analysis considering the type of DNA tests, patient characteristics, and treatment type did not modify this conclusion. Currently sperm DNA testing lacks sufficient predictive power to effectively inform routine treatment but may be helpful in couples with unexplained recurrent ART or pregnancy failure seeking an explanation. Wider application of sperm DNA integrity testing requires the development of better assays, *e.g.* in allowing simultaneous assessment sperm vitality and TUNEL as recently described (102), and data from adequately powered prospective studies relating sperm DNA data to outcomes in severe male factor infertility. Data supporting the effectiveness of medical therapy (*e.g.* antioxidant/vitamin therapy) from adequately powered placebo-controlled randomized controlled trials and/or the ability to isolate less damaged sperm would strengthen this field (103).

Androgen receptor (AR) mutations

Several hundred mutations of *AR* have been described with resultant phenotypes ranging from testicular feminization to partial androgen insensitivity syndrome to male infertility. The *AR* has an essential role in transducing androgen action on spermatogenesis, and whereas missense mutations have been associated with an isolated male infertility phenotype (104), the prevalence rate is low and assessment is rarely performed. Clinical presentations indicative of subtle *AR* mutations include clinical evidence of androgen deficiency despite raised serum LH and testosterone levels. Of much greater controversy, exon 1 of *AR* is involved in receptor transactivation and the length of its CAG repeat sequence varies inversely with receptor activity, and expansions of the CAG repeat length in the moderate range, have been associated with azoo-oligospermia (105). Since this first report, many others have supported and refuted this association (106). Suffice to say that any relationship between sperm density and CAG repeat length is weak and applicable to only some racial

groups, the implications for counseling are uncertain and its routine assessment is not recommended.

Sperm functional defects

The presence of immotile but viable sperm raises the possibility of primary ciliary dyskinesia, a heterogeneous syndrome with a live birth frequency of one in 20,000–60,000 (MIM no. 242,650) characterized by varying degrees of respiratory tract dysfunction, infertility, heterotaxia, or *situs inversus* totalis (also called Kartagener's syndrome) and hydrocephalus (107). To date mutations in six different genes have been associated with primary cilia dyskinesia in humans: *DNAI1*, *DNAI2*, *DHAH5*, and *DNAH11*, all of which encode axonemal dyneins; *TXNDC3*, which encodes a thioredoxin; and *RPGR*, which is an X-linked gene also associated with retinitis pigmentosa (108–112). Collectively, these mutations account for less than 40% of primary ciliary dyskinesia cases, of which *DNAH5* mutations (15%) are most common (113, 114). The majority of patients do have sperm in the ejaculate, and sperm have been successfully used in ICSI (115). Patients may first present with severe asthenospermia because other features have variable penetrance, but the presence of respiratory features and/or dextrocardia increases the likelihood of primary ciliary dyskinesia.

Recently mutations in the aurora kinase C (*AURKC*) gene have been shown to cause infertility in men from the Maghreb region of Africa (including Morocco, Tunisia, and Algeria) (116) wherein a carrier frequency of one in 50 has been reported (117). Men homozygous for c.144delC *AURKC* mutations produce sperm with a 4N chromosomal complement, large heads, and often multiple tails. As yet there are no data to support the screening in other populations or sperm phenotypes such as common forms of teratospermia. Interestingly, *Aurkc* knockout mice, although showing a high rate of teratospermia, retained fertility (118).

Emerging genetic considerations in male infertility

Much attention is now being paid to the relationship between apparently small changes in genomic sequence and male infertility. We highlight three areas that may, within the next few years, impact fertility practice and ART-conceived offspring (Fig. 3).

Copy number variations (CNVs)

CNVs are defined as pieces of DNA, 1 kb or longer, that vary in number between individuals. Simplistically they represent submicroscopic duplications and/or deletions of the genome (119). CNVs can cause overt disease (pathogenic CNVs), a predisposition to disease, or apparently have no effect (benign CNVs). CNVs sit at the interface

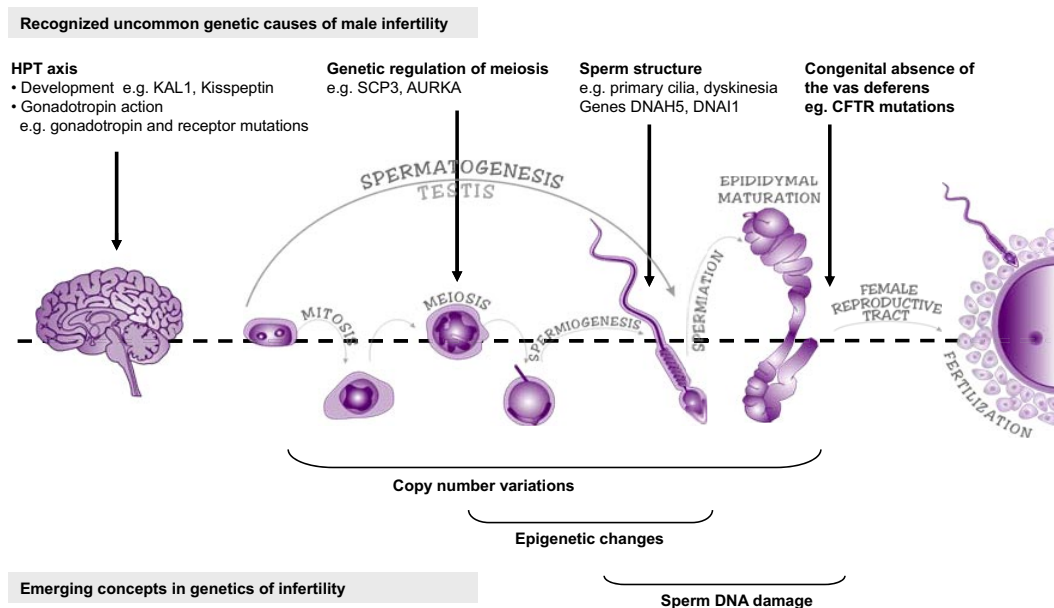


FIG. 3. Known, but rare, and emerging causes of human male infertility. Areas of research below the dotted line are increasingly being implicated in male infertility, although the precise mechanisms and genes involved with infertility are yet to be discovered. See text for discussion. HPT, Hypothalamic-pituitary-testis.

between microscopically visible rearrangements and point mutations and are increasingly being assessed, using microarray or PCR-based methods, in the investigation of diverse conditions including autism, schizophrenia, and congenital adrenal hyperplasia (120–122). Indeed recent studies have cumulatively shown that CNVs affect about 20% of the human genome (<http://projects.tcag.ca/variation>). With the exception of changes in Y chromosome numeric gene content, CNVs have not yet been definitively defined as a cause of male infertility, but that seems inevitable. Technologies to allow the assessment of single cells for CNV, for preimplantation genetic diagnosis, are already under development (123).

Epigenetic changes

Whereas not strictly genetic, changes in the human epigenome are increasingly being associated with human male infertility. Epigenetic mechanisms include the way in which the genome is packed and thus the ability for genes to be activated. Epigenetic changes can be inherited across cell divisions or across generations and can have a profound effect on an individual's phenotype. It is clear that homozygous mutations in key epigenetic regulators affect male fertility more overtly than most biological systems (124, 125) and that sperm from some infertile men have an abnormal epigenome (126, 127). Data from animal models show that the sperm's epigenome is subject to environmental influences (128, 129) and that changes in the father's epigenome can have consequences for the phenotype of his offspring, even in the absence of inheritance of the causal mutation (130). At present in humans,

there are no data relating the epigenetic marking of individual genes in sperm and subfertility, but such associations are likely and may have important implications for public health recommendations and ART practice.

Point mutations in spermatogenesis genes

It is estimated that more than 2300 genes are involved in male fertility (131). As of mid-2008, approximately 400 of these genes had been modeled in mice (1). With the full implementation and scaling up of the international mouse knockout program (132) and several large-scale random mutagenesis programs (133), however, our knowledge of genotype-phenotype correlations will accelerate at an exponential rate. Whereas it is true that the translation of knowledge gleaned from mutations in mice to the fertility clinic has been frustratingly slow, these are the genes that are likely to contribute to multigenic infertility and to be causal in subtle genomic variations, such as the CNVs and epigenetic changes discussed above.

Conclusions

Recognized genetic disorders account for an important minority of presentations with primary spermatogenic failure or obstructive azoospermia, and routine karyotype and Y microdeletion analyses and *CFTR* screening are indicated, respectively, as outlined. Within certain families and clinical settings, a wider range of other tests are also relevant. Diagnosis is essential to ensure proper counseling about the effectiveness and safety of ART treatment and amelioration strategies such as preimplantation genetic diagnosis. Over time, new genetic lesions will be

identified and more complete, rapid, and hopefully cheaper testing strategies will become available. For this reason, fertility clinics must remain actively engaged with geneticists in translational research so that developments can be rapidly and effectively implemented.

Acknowledgments

We thank Csilla Krausz, Elissa Osborne, Stephanie Smith, and Claire Borg for their kind assistance in the review of the manuscript and preparation of the figures.

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R.I.M. and M.K.O. are funded by fellowships from the National Health Medical Research Council of Australia.

Disclosure Summary: The authors have nothing to disclose.

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