Genetic Diagnosis in Male Infertility

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Clinically, the most common genetic factors associated with male infertility are chromosomal abnormalities, Y chromosome deletion, and cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations that are associated with congenital bilateral absence of vas deferens (CBAVD). The prevalence of chromosomal abnormalities in all infertile men is 5–7%. Patients with azoospermia are likely to possess sex chromosome abnormalities, and Klinefelter syndrome (47,XXY) is the most prevalent sex chromosome disorder. Conversely, oligozoospermic men are likely to have autosomal chromosome abnormalities, which include chromosomal translocation and inversion. Y chromosome deletion is the second most frequent genetic cause of spermatogenic failure after Klinefelter syndrome. The current deletion model, which has been revised according to the recombination mechanisms, includes AZFa, P5/proximal P1, P5/distal P1, P4/distal P1 and AZFc (b2/b4) deletions. The overall deletion frequency in Taiwanese infertile men is 6.5%, and AZFc (b2/b4) deletion is the most common deletion pattern, which does not show an ethnicity-specific deletion pattern in the Taiwanese population. CBAVD accounts for ~2% of male infertility and ~6% of obstructive azoospermia. It has been shown that CBAVD is associated with CFTR gene mutation. The mutation spectrum of the CFTR gene in Taiwanese CBAVD patients is different from that of the Caucasian population; the Taiwanese do not carry the common CFTR mutations found in Caucasians. Polymorphic polythymidine tract in intron 8 5T allele accounts for the majority (81%) of the mutant alleles in Taiwanese CBAVD. In conclusion, suggestions for current genetic testing in male infertility are as follows: karyotype analysis should be offered to all oligozoospermic men; an additional Y chromosome deletion test is recommended for men with severe oligozoospermia (<5 x 10⁶/mL) or non-obstructive azoospermia; and detection of CFTR gene mutation status is necessary for men with structural abnormalities of the vas deferens. Genetic testing can be helpful for reproductive decision making by physicians as well as infertile couples, which might obviate the need for assisted reproductive techniques and the production of congenital defects in the offspring.

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There are 2 CME questions based on this article

1. Introduction

Advances in the intracytoplasmic sperm injection (ICSI) technique have resulted in many infertile men achieving paternity using a limited number of sperm. However, the majority of couples in which the men have severe oligozoospermia or non-obstructive azoospermia still cannot achieve pregnancy even with ICSI.¹ Possible reasons for
this are that the causes of male infertility are unknown in up to 25–30% of cases, and that most cases of idiopathic male factor infertility are believed to be genetic in origin.\textsuperscript{2–5} In contrast, it has been shown that an increase in congenital abnormalities in infants born after ICSI is more likely to be linked to the injection of genetically abnormal sperm.\textsuperscript{6} Therefore, genetic factors are frequently associated with spermatogenic failure and should play a crucial role in male infertility. Although our knowledge of the genetic causes of male infertility has increased in the past decade, advances in the genetic diagnosis of male infertility have been relatively limited. To date, the three most common genetic factors that are related to male infertility are numerical or structural chromosomal abnormalities, Y chromosome deletion, and cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations that are associated with congenital absence of vas deferens.

2. Chromosomal Abnormalities

2.1. Male infertility and chromosomal abnormalities

Chromosomal abnormalities can be divided into sex and autosomal chromosome abnormalities. It has been estimated that the prevalence of chromosomal abnormalities in infertile men is approximately 5.8%, with prevalence of sex chromosome abnormalities at 4.2% and autosomal chromosome abnormalities at 1.5%.\textsuperscript{7} An analysis of neonatal boys has shown that the prevalence of chromosomal abnormalities is 0.38%, with prevalence of sex chromosome abnormalities at 0.14% and autosomal chromosome abnormalities at 0.25%.\textsuperscript{5} Other studies have shown that the prevalence of chromosomal abnormalities is 5% for infertile men with oligozoospermia and 10–15% for those with azoospermia.\textsuperscript{8,10} and that, among all couples who undergo ICSI, 4.3% of the men have chromosomal abnormalities.\textsuperscript{11} Generally, azoospermic men are likely to possess sex chromosome abnormalities, and Klinefelter syndrome (KS) is the most prevalent sex chromosome disorder. In contrast, oligozoospermic men are likely to have autosomal chromosome abnormalities, which include chromosome translocation and inversion.\textsuperscript{12,13} With the increasing severity of spermatogenic failure, the possibility that the chromosome is abnormal also increases. Therefore, all infertile couples who seek in vitro fertilization or ICSI because of spermatogenetic defects should receive standard chromosome karyotype analysis.

2.2. Klinefelter syndrome (47,XXY)

Klinefelter syndrome is not unusual in the male infertility clinic. The incidence of KS is approximately 1 in 500 (0.2%) among the male population, and it is the most common sex chromosome abnormality.\textsuperscript{14} The extra X chromosome can induce spermatogenic as well as steroidogenic failure, which results in testicular atrophy, azoospermia and gynecomastia. However, KS patients can present with a broad spectrum of phenotypes, professions, and socioeconomic statuses.\textsuperscript{15} The most common clinical characteristics are small testes, elevated serum follicle-stimulating hormone level, and low or low-to-normal testosterone levels. Despite a low testosterone level, a patient’s libido is generally normal.\textsuperscript{15} However, KS patients might require androgen replacement therapy in later adulthood, because the testosterone level tends to decrease with age.

Approximately 3–10% of KS is mosaic form (46,XY/47,XXY), and more than half of the patients still have spermatogenesis in their testes. Conversely, the remaining 90% of KS is non-mosaic 47,XXY, and most patients present with azoospermia. However, focal spermatogenesis might still exist in the atrophic testes of non-mosaic KS patients. Recent reports have demonstrated that 21–69% of non-mosaic KS patients might have successful testicular sperm extraction (TESE),\textsuperscript{16,17} and younger patients (age <35 years) might have a better chance of having spermatozoa.\textsuperscript{18} Consistent with these observations, Damani et al.\textsuperscript{19} have suggested a strategy of early TESE and sperm cryopreservation at puberty, given that spermatogenesis actively begins at this time and is gradually lost as long as the compromised testicular environment continues.

The incidence of sex chromosome abnormalities in sperm retrieved from KS patients ranges from 0.9% to 7.5%, compared with 0.4% in normal controls.\textsuperscript{20,21} In addition, the incidence of sex chromosome abnormalities in the embryo is only 3.1% in controls, compared with 13.2% in KS patients.\textsuperscript{22} These results highlight the need for genetic counseling and preimplantation genetic diagnosis in these patients. Nevertheless, one recent review has revealed that only one KS karyotype in one fetus was noted among 39 reported successful pregnancies fathered by non-mosaic KS patients.\textsuperscript{23}

2.3. 46,XX male syndrome

46,XX men are relatively rare in male infertility clinics, and the incidence is approximately 1 per 20,000 among the general population.\textsuperscript{19,24,25} In 90% of the 46,XX males, the paternal sex-determining region Y (SRY) gene is translocated to the X chromosome, or rarely, to the autosome.\textsuperscript{26} The other 10% of the SRY-negative 46,XX males are believed to have mutations/polymorphisms or gene expression defects, along with female gonadal development, which results in testicular differentiation of bipotential gonads and testosterone secretion.\textsuperscript{24} Testosterone is converted into dihydrotestosterone via the action of 5α-reductase, which results in the development of male external genitalia. Given the lack of Y chromosome containing the spermatogenesis-related genes, germ cells are not present in the testes of 46,XX males. Thus, neither diagnostic testicular biopsy nor TESE is recommended for these patients.
2.4. Chromosomal translocation and inversion

Chromosomal translocation accounts for 2–3% of male infertility. To date, over 50 different chromosomal translocations have been reported to be associated with spermatogenic failure. Robertsonian translocation of chromosomes 13 and 14 is well known to result in oligoasthenoteratozoospermia.27 Certain translocations can interfere with prophase I pairing in meiosis and disrupt its progression, thus the patients might present with severe oligozoospermia or azoospermia.

Chromosomal inversion can be divided into pericentric (around the centromere) or paracentric (beyond the centromere) inversion. If the genetic material is not lost, gained or altered by the inversion, there is no obvious effect on the phenotype of the individual. However, chromosomal inversion could interfere with chromosome pairing in prophase I of meiosis, which results in disruption of meiosis, and subsequently, oligozoospermia or azoospermia. Loss or duplication of the genetic material produces abnormal gametes. Therefore, the risk of miscarriage and children born with chromosomal and congenital defects is high. Amniocentesis and preimplantation genetic diagnosis can be used preemptively to increase the chance of transferring only chromosomally normal embryos.28

3. Y Chromosome Deletions

3.1. Y chromosome deletion model

The human Y chromosome contains many amplicons that are non-X-transposed, non-X-degenerated, repetitive sequences with nearly complete identity in male-specific regions. Non-allelic homologous recombination between amplicons has been shown to generate deletions with resultant spermatogenic failure.29–31 Deletions of azoospermatia factors (AZFs) on the long arm of the Y chromosome (Yq) have been widely studied and recognized as the second most frequent genetic cause of spermatogenic failure after KS.32 These genes are located in the proximal, middle and distal subregions of Yq11, and are designated as AZFa, AZFb and AZFc, respectively.33 Recently, the deletion models have been revised according to the recombination mechanisms identified between palindromes P5 and P1.34,35 These are AZFa, P5/proximal P1, P5/distal P1, P4/distal P1, and AZFc (b2/b4).

3.2. Detection of Y chromosome deletions

The European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) have recommended a minimal set of primers for screening Y chromosome deletions, which includes six sequence-tagged sites as a basic set of primers for diagnosis: sY84 and sY86 for AZFa, sY127 and sY134 for AZFb, and sY255 for AZFc, with SRY and ZFY/ZFX as controls. The EAA/EMQN best practice guidelines state that this primer set enables the detection of all the clinically relevant deletions and >95% of the deletions in the three AZF regions.36 The EAA/EMQN protocol is widely accepted and supported by the American Urological Association, American Association of Bioanalysis, American Society for Reproductive Medicine, United Kingdom Human Fertilisation and Embryo Authority, and French Society for Human Genetics.

3.3. Y Chromosome deletion status in Taiwan

In our institute, a gene-specific, five-marker panel is used to detect the Y chromosome deletion status in Taiwanese infertile men. From 2000 to 2005, a total of 627 infertile men and 212 proven fertile controls were tested. Forty-one patients were found to have Y deletions. These patients included three with AZFa (7%), eight with AZFb+c (20%), and 30 with AZFc deletions (73%).37,38 We also used a triple-blind study design to compare the efficacy of our five-marker panel protocol and the EAA/EMQN protocol. The results showed a consistent deletion status between our protocol and that of the EAA/EMQN. Moreover, a review of >4800 infertile patients has shown that Y chromosome deletion most frequently involves the AZFc region (60%), less frequently the AZFb region (25%), and least of all the AZFa interval (5%).39 Taken together, our data do not show an ethnicity-specific deletion pattern of the Y chromosome in the Taiwanese population. AZFc (b2/b4) deletion was the most common deletion pattern in our series and others. The AZFc region is composed completely of amplicons, and homologous recombination between amplicons b2 and b4 is probably one of the most common genetic causes of spermatogenic failure.40

The overall deletion frequency in our series was 6.5% (41 of 627). The deletion rate was 11.6% (34 of 293) in patients with sperm concentration <10^6/mL or azoospermia, 4.5% (5 of 110) in patients with sperm concentration between 1×10^5 and 5×10^5/mL, and 0.9% (2 of 224) in patients with sperm concentration >5×10^5/mL. Our findings are consistent with previous reports that Y chromosome deletions are found almost exclusively in patients with sperm concentration <10^6/mL, and are extremely rare in those with a sperm concentration >5×10^5/mL.39,40 Given the low deletion rate (0.9%) in this series and in others (0.7%),41 it is reasonable to set the threshold for Y chromosome deletion testing at <5×10^5/mL or non-obstructive azoospermia.

3.4. Genotype–phenotype correlation

In some studies including our own, attempts have been made at genotype–phenotype correlation, which have produced a general rule. Deletions of the AZFa, P5/proximal P1, P5/distal P1, P4/distal P1 regions are always associated...
with the Sertoli cell-only syndrome testicular phenotype. These patients might not obtain any benefit from testicular sperm retrieval. Conversely, deletion of the AZFc region can lead to oligozoospermia, testicular hypospermatogenesis or maturation arrest of postmeiotic germ cells. It is always possible to find sperm in the testes. Therefore, Y chromosome deletion status might be used as a predictor of successful sperm retrieval in infertile men.

4. Congenital Bilateral Absence of Vas Deferens and CFTR Gene Mutations

4.1. Congenital bilateral absence of vas deferens, cystic fibrosis and CFTR gene

Congenital bilateral absence of vas deferens (CBAVD) accounts for ~2% of cases of male infertility and ~6% of infertile men with obstructive azoospermia.\(^4^2\) It has also been reported that CBAVD is present in approximately 95% of male patients with cystic fibrosis.\(^4^3\) From multiple surveys in Caucasian populations, ~66% of men with CBAVD carry mutations in the CFTR gene.\(^4^4,4^5\)

Cystic fibrosis is a lethal autosomal recessive genetic disorder in Caucasians, which is characterized by chronic pulmonary inflammation, pancreatic exocrine insufficiency, elevated concentrations of electrolytes in sweat, chronic sinusitis, diabetes mellitus, liver cirrhosis, and CBAVD.\(^3^6\) The CFTR gene is located on chromosome 7 (7q31–q32) and contains 27 exons. To date, >1000 mutations of CFTR genes have been found, and over two-thirds of mutant alleles in cystic fibrosis patients are ∆F508 (in-frame deletion of phenylalanine at residue 508) and R117H (missense mutation in exon 4 with Arg117-to-His).\(^4^6–4^8\) However, the routine mutation testing recommended by the American College of Medical Genetics for cystic fibrosis patients is not adequate to detect the mutation status in CBAVD patients.\(^4^9\) To date, a number of published series in Caucasians have shown that polymorphic polythymidine tract in intron 8, in addition to ∆F508 and R117H, is associated with CBAVD phenotype.\(^4^6,5^0–5^4\)

4.2. Screening of CFTR mutation in Taiwan

The mutation spectrum of the CFTR gene in Taiwanese CBAVD patients has been extensively analyzed by Taipei Medical University Hospital.\(^5^5,5^6\) The results have shown that Taiwanese CBAVD patients do not carry any of the common CFTR mutations found in Caucasians, and that seven homozygous and seven heterozygous 5T alleles in the intron 8, polythymidine tract are present. The 5T allele accounts for the majority (81%) of the mutant alleles in Taiwanese CBAVD. In addition to the polythymidine tract in intron 8, five mutations, including p.V201M, p.N287K, c.–8G>C (125G>C), p.M469I and p.S895N, have been found in five patients, and p.N287K, p.M469I and p.S895N are novel mutation sites. The overall frequency of CFTR mutant alleles in Taiwanese men with CBAVD is 36% (26 out of 72 alleles).

4.3. Risk of cystic fibrosis in offspring

With the development of assisted reproductive technique, infertile men with CBAVD are able to achieve paternity by using sperm retrieval and ICSI. CBAVD is linked to CFTR gene mutation; therefore, offspring have an associated increased risk of cystic fibrosis. The European Association of Urology guidelines on male infertility confirm this probability. For a man with CBAVD, if his partner is found to be a carrier, the chance of a baby with cystic fibrosis is 25% if he is heterozygous or 50% if he is homozygous. If his partner is negative for unknown mutations, her chance of being a carrier of an unknown mutation is about 0.4%, and the possibility of her heterozygous partner fathering a child with cystic fibrosis is approximately 1 in 410.\(^5^7\) Therefore, the detection of CFTR gene mutation status is crucial for a man with CBAVD and his partner prior to sperm retrieval and ICSI.

5. Conclusion

Standard karyotype analysis should be offered to all men with oligozoospermia or non-obstructive azoospermia who are seeking fertility treatment. For men with severe oligozoospermia (<5 × 10⁶/mL) or non-obstructive azoospermia, testing for Y chromosome deletion prior to ICSI is recommended. When a man has structural abnormalities of the vas deferens (CBAVD or unilateral absence of the vas), it is important to test the couple for CFTR gene mutations. Genetic counseling is mandatory for all couples with genetic abnormalities, which might obviate the need for TESE, and could ultimately alter the plan for assisted reproductive techniques.

References

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