Recent advances in the genetics of testicular failure

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INTRODUCTION

Testicular or spermatogenic failure is the most severe form of male infertility. The typical phenotype of testicular failure is severely impaired spermatogenesis resulting in azoospermia or severe oligozoospermia (sperm density ≤5 × 10^6 ml^-1). A variety of conditions, both congenital and acquired, can cause testicular failure. However, even after a complete diagnostic work-up, in about 40%–50% of primary testicular failure, the etiology remains unknown. This is mainly due to the lack of understanding of all the molecular and genetic mechanisms responsible for male fertility defects. Despite several advances in the identification of genetic defects that lead to infertility in animal models, the scope of clinical testing for genetic defects in men with infertility remains narrow.

Male factor infertility comprises about 30%–50% of all infertility, and genetic abnormalities are thought to account for 15%–30% of male factor infertility. Increasing utilization of assisted reproductive techniques (ARTs), especially intracytoplasmic sperm injection (ICSI), has potentially increased the risk of transmission of genetic abnormalities to the offspring. Recently, along with other intense researches ongoing, whole-genome approaches have been used increasingly in the genetic studies of male infertility. In this review, we focus on the genetics of testicular failure and provide an update on the advances in the study of genetics of male infertility.

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GENETIC CAUSES OF TESTICULAR FAILURE

Human spermatogenesis, which takes about 3 months, depends on the co-ordinated action of thousands of testis-specific genes for satisfactory completion of meiosis. It is a complex series of very delicate events vulnerable to the accumulation of errors that could affect the whole spermatogenic process resulting impaired spermatogenesis ranging from oligozoospermia to complete azoospermia. It is well-known that there is a genetic etiology in many cases of male infertility. Several genetic abnormalities associated with testicular failure are briefly summarized below.

Abnormalities of sex chromosomes

Chromosomal abnormalities account for approximately 6% of infertility in men, and the prevalence increases to 15% among men with azoospermia. Aneuploidy, or incorrect chromosome number, is the most common error resulting from chromosomal abnormalities in infertile men. Men with nonobstructive severe oligozoospermia or azoospermia have a particularly high incidence of aneuploidy, particularly in their sex chromosomes. Klinefelter syndrome (KS) or 47,XXY is the most common sex chromosome aneuploidy in human males with two or more X chromosomes, occurring in approximately 1:500 newborn males. Men with KS have primary testicular failure secondary to the extra X chromosome. There are two forms of KS: nonmosaic, 47,XXY; and mosaic, 47,XXY/46,XY. The extra X chromosome in men with KS is retained because of a nondisjunction event during paternal or maternal meiosis I.

A recent review showed that a small number of mature spermatozoa could be detected in some of these patients for possible use in IVF/ICSI. In addition to fertility issues, two-thirds of KS patients have low serum androgen levels and exhibit hypogonadal symptoms such as osteoporosis, gynecomastia, and poor erectile function. Given
the high incidence of KS and hypogonadal symptoms, long-term androgen replacement and regular follow-up are recommended in KS patients.19,20

XYY is another sex chromosomal abnormality occurring in approximately 1:1000 live male births.21,22 Parental nondisjunction at meiosis II resulting in an extra Y chromosome produces a 47,XYY karyotype in the affected offspring.23,24 Most men with XYY are phenotypically normal, except for increased height. Men with 47,XYY syndrome can have variable sperm counts, ranging from normal to azoospermia. Although fertility may vary in XYY men, studies have reported an increased incidence of chromosomally abnormal spermatozoa in their semen.25,26

46,XX testicular disorder of sex development (also known as 46,XX male syndrome) occurs in approximately 1:20 000 newborn male babies.27 Ninety percent will have sex-determining region Y (SRY, located on Yp) translocated to either the tip of the X chromosome or to an autosome. The remaining SRY-negative 46,XX men are presumed to have abnormalities somewhere along the genetic cascade.28 These XX male patients are azoospermic with Sertoli cell-only pathology on testis biopsy. Therefore, men with 46,XX should not undergo testicular sperm extraction (TESE).

Chromosomal translocations
Translocations are another common source of chromosomal aneuploidy. Chromosomal translocations may cause the loss of genetic material at the breakpoints and could result in testicular failure.29 There are two types of translocations: reciprocal translocation and Robertsonian translocation. Chromosomal translocation is to be found 4-10 times more commonly in infertile males than in normal males.30 Robertsonian translocation occurs when two acrocentric chromosomes fuse with an incidence of approximately 1:1000 and may affect spermatogenic process.31 Previous studies showed that chromosomal translocation in men can cause meiotic disturbance during spermatogenesis and produce sperm with superabundant or insufficient genetic material. Embryos derived from sperm with unbalanced genetic material would not develop appropriately.32,33 Therefore, fertilization with sperm from a father who has a chromosomal translocation defect can lead to recurrent miscarriage or genetic abnormality in offspring. Genetic counseling and, possibly IVF/ICSI, in combination with preimplantation genetic diagnosis (PGD) should be recommended to ensure that the offspring is not aneuploid.34

Y chromosome microdeletion
Y chromosome microdeletions are among the best known genetic causes of male infertility.35-38 Y chromosome microdeletions are found in 10%-15% of men with nonobstructive azoospermia or severe oligospermia.39,40 A microdeletion is a chromosomal deletion that spans several genes but is not large enough to be detected using conventional cytogenetic methods. The Y chromosome is approximately 60 million base pairs in length, equally divided between the euchromatic and heterochromatic regions, and contains many of the genes that are critical for spermatogenesis and the development of male gonads.

Microdeletions most frequently occur on the long arm of the Y chromosome (Yq), and deletions in this region are specifically related to the failure of spermatogenesis. The first Y chromosome microdeletion was reported by Tiepolo and Zuffardi in 1976, when they observed a deletion in Yq11 in patients with azoospermia.41 On the basis of these observations, they proposed the existence of a gene involved in spermatogenesis, named Azoospermia Factor (AZF). The AZF region contains three sub-regions: AZFa, AZFb, and AZFc (Figure 1).

Aberrant homologous recombination between Y chromosome palindrome structures has been suggested as an underlying mechanism of Y chromosome microdeletion.42 A palindrome is a segment of DNA in which the nucleotide sequence in one strand read from one end is the same as the sequence in the complementary strand read from the opposite end, and Y chromosome palindromes consist of mirror image DNA segments that allow for local recombination (Figure 2).

AZFa
The AZFa region, which is located in proximal Yq, is 792 kilobases (kb) in length. It differs from AZFb and AZFc because of its nonrepetitive structure and its low deletion frequency (0.28% among men with nonobstructive azoospermia).36 Two main genes located in the AZFa region are USP9Y and DBY (also called DDX3Y). 43 DBY is the major gene located in the AZFa region, and its product is localized in the testis and involved in the development of premeiotic germ cells.44 The USP9Y gene is also involved in spermatogenesis. Shortening or deletion of the USP9Y gene causes azoospermia or oligozoospermia.45 It has been reported that deletions in the AZFa region that remove these genes and loss of these two genes result in the absence of spermatogenesis or Sertoli cell-only syndrome.46 Therefore, TESE is not recommended to men with complete deletions in the AZFa region due to lack of identifiable regions of spermatogenesis. Partial deletion of AZFa (USP9Y gene) can result in oligozoospermia.47

AZFb
The main gene in the AZFb region is RBMY, and there are six copies of the gene located on the Y chromosome.48 AZFb deletions result in the loss of RBMY1 which codes a unique testis-specific splicing factor and PRY, a gene involved in the apoptotic pathway, then lead to maturation arrest at the primary spermatocyte stage.49 A family of PRY genes is also found in the AZFb region of the Y chromosome. The PRY genes are involved in the regulation of apoptosis, an essential process that removes abnormal sperm from the population of spermatozoa. It is reported that spermatogenesis is arrested completely, if both the RBMY and PRY genes are removed.50 TESE is not recommended to
men with complete deletions in the AZFb region owing to testicular histology of uniform maturation arrest and lack of identifiable regions of spermatogenesis.

AZFc

The AZFc is the most commonly microdeleted region, found in 13% of azoospermic and 6% of severely oligozoospermic men. The AZFc region also contains important genes involved in spermatogenesis, such as the DAZ gene which has four copies on the Y chromosome. DAZ genes are thought to serve a variety of roles throughout the spermatogenic process because they are expressed in all stages of germ cell development. Deletions of the DAZ genes can cause a spectrum of phenotypes ranging from oligozoospermia to azoospermia. Whereas the AZFa and AZFb deletions are regarded as very specific, the AZFc microdeletions are more heterogeneous ranging from intra-chromosomal recombinations and sub-deletions to complete deletions of the intervening region (Figure 1). The three most frequently found sub-deletions on the AZFc region of the Y chromosome are gr/gr, b1/b3, and g1/g3 (b2/b3). Deletions in the AZFc region also produce a wide range of phenotypes, many of which are associated with low sperm concentration due to impaired spermatogenesis.

Clinical implications of Y chromosome microdeletion

Many cases of severe male infertility are caused by deletions of Y chromosome and Y chromosome microdeletion has been known to be transmitted to male offspring. Concern has been raised that if they carry the same genetic defects as their father’s on their Y chromosomes, they can be infertile as their fathers were. Since ICSI has been performed from 1992, especially for couples with severe male factor infertility, the first young men born from ICSI procedure are about to get married. Therefore, it would be prudent to recommend early fertility evaluation for these men. The residual spermatogenic function in some infertile men with Y chromosome microdeletions has been observed to decrease over a period of time. Early sperm cryopreservation could be considered before spermatogenesis completely deteriorates.

The genotype–phenotype correlation has another important clinical implication. Since the initial identification of AZF on Yq, many studies have suggested the prognostic value of specific Y chromosome microdeletions. It is generally assumed that the identification of sperm in men with AZFa or AZFb region deletions is unlikely, whereas men with AZFc deletions have a higher chance (up to 70%) of having sperm identified on surgical sperm extraction. However, the functional contribution of genes from the deleted region to spermatogenesis is still not properly investigated, and there are significant differences among the various studies regarding the type and prevalence of deletions. Therefore, a strict genotype–phenotype correlation is still insufficient, and the type of AZF deletion alone may not be proposed as a potential prognostic factor for sperm retrieval.

Most laboratories use a polymerase chain reaction (PCR) assay for amplification of specific regions of the Y chromosome, named sequence-tagged sites (STSs). Sequence-tagged sites are short segments of DNA that function as markers for specific loci on the genome. The Y chromosome contains 300 STSs. Because different laboratories use different STSs, the test results might not be the same among laboratories. The assay should include STSs that span the AZF region as well as other regions thought to code for putative spermatogenesis genes. It is critically important that the PCR assay should be performed with both positive and negative control. Laboratories should follow a standard protocol to improve the sensitivity and accuracy of PCR diagnoses of Y chromosome microdeletions.

Other genetic causes of testicular failure

Hypogonadotropic hypogonadism (HH) is another important cause of male infertility that can lead to testicular failure associated with underlying genetic defects. In the past 20 years, we have witnessed an explosion in the knowledge of hypotalamo-pituitary development. A complex genetic cascade dictates organ commitment, cell differentiation, and cell proliferation within the anterior pituitary (Figure 3). Without adequate levels of gonadotropins, androgen production and spermatogenesis fail.

Kallmann syndrome is defined as idiopathic hypogonadotropic hypogonadism (IHH) combined with anosmia or hyposmia that can cause testicular failure. Men with Kallmann syndrome have both X-linked and autosomal genetic defects. The disorder is caused by a defect in the migration of the GnRH neurons. IHH is characterized by low levels of sex steroids in combination with low levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Two genetic deletions found in men with Kallmann syndrome are KS1 sequence (KAL1) and fibroblast growth factor receptor 1 (FGFR1). KAL1, located on the short arm of the X chromosome, is involved in the migration of the GnRH neurons, and it codes for anosmin-1, a cell adhesion molecule. KAL1 mutations are thought to be responsible for 30%–70% of KS in patients. Deletion of the FGFR1 gene on chromosome 8 can cause either anosmic or hyposmic forms of Kallmann syndrome. Hormonal replacement therapy with hCG...
and/or recombinant FSH for IHH generally shows good response in the recovery of spermatogenesis.

Congenital hypopituitarism with multiple pituitary hormonal defects can also be associated with hypogonadotropic hypogonadism resulting in testicular failure. Mutations in genes encoding both signaling molecules and transcription factors have been implicated in the etiology of hypopituitarism, with or without other syndromic features, in mice and humans. These include HESX1, LHX3, LHX4, PROP1, POUIF1 and, more recently, SOX3 and SOX2.45

Leydig cell hypoplasia is another rare cause of testicular failure associated with the underlying genetic defect. Human male sexual development is regulated by chorionic gonadotropin and luteinizing hormone (LH), the action of both is mediated by the LH receptor (LHR). Mutations that inactivate the LHR cause Leydig cell hypoplasia, an autosomal recessive disorder. In its mild form, Leydig cell hypoplasia patients present with male hypogonadism. In its severe form, patients present with male pseudohermaphroditism, with female external genitalia, and cryptorchid testis, resulting in male infertility.56

**GENETIC CAUSES OF OBSTRUCTIVE AZOOSPERMIA**

Congenital bilateral agenesis of vas deferens (CBAVD) is found in approximately 1% of infertile males and is one of the common diagnoses in patients with obstructive azoospermia.57 Cystic fibrosis represents one of the two main genetic etiologies for CBAVD.58 It is an autosomal recessive disease that occurs in 1:1600 people of Northern European descent, and about one in 25 people are carriers. Although cystic fibrosis is a relatively common genetic disease in Western countries, the incidence of cystic fibrosis is very rare in other regions such as East Asia showing racial differences. The CF gene is located on chromosome 7 and the gene product is a transmembrane protein termed CFTR, which regulates the viscosity of epithelial secretions in the respiratory system. Men with CBAVD usually have either two mild mutations in the CFTR gene or the combination of a severe mutation and a mild mutation. More than 1000 CFTR mutations have been identified. In addition, there is a polymorphism in intron 8 of CFTR gene that quantitatively influences the production of CFTR protein. The alleles of the polymorphic region of thymidine in intron 8 contain five (5T), seven, or nine thymidine. The 5T allele is associated with reduced levels of functional CFTR protein.59,60

Spermatogenesis is mostly normal in CBAVD patients, and sperm can be recovered from the epididymides or testes for IVF/ICSI. CF mutation analysis is recommended in all CBAVD patients, and partner screening is critical to the couple. When both partners carry the mutation, they should be advised to have PGD to avoid passing the abnormality to their offspring.60-72

**CURRENT STATUS**

Spermatogenesis is an ongoing developmental process in adult testes that requires the co-ordinated expression of many genes. The phenotype of testicular failure is severely impaired spermatogenesis resulting in azoospermia or severe oligozoospermia, commonly requiring ART treatment for pregnancy. By virtue of research in the last decades, current genetic tests, including karyotyping and Y chromosome microdeletion testing, are widely available and of direct benefit to patients. Correct genetic diagnosis is essential to ensure proper counseling among the infertile couples about the effectiveness and safety of ART treatment such as IVF/ICSI and preimplantation genetic diagnosis. Recent investigations have enabled the identification of many genes involved in spermatogenesis, and their mechanisms of action are currently being clarified. Although most researches in the genetics of male infertility have focused on the Y chromosome, studies indicate that the Y chromosome is not the only chromosome that accumulates genes which benefit spermatogenesis. It has been predicted that more than 2000 genes are involved in spermatogenesis.63 Many X-linked genes are expressed in the testis and are thought to be involved in spermatogenesis, and autosomal genes are also being investigated as candidate genes for possible roles in male factor infertility.64,65

Unfortunately, despite substantial efforts over the last decades, our understanding of the genetic contribution and mechanism of testicular failure still largely remains preliminary. Although many potential candidate genes have been proposed to be involved in male infertility, their causative effects largely remain to be proven. This is partly because spermatogenesis is a highly sophisticated process involving complex molecular pathways requiring hundreds of genes that is vulnerable to the accumulation of defects such as meiotic errors that could affect the whole spermatogenic process. For men with unexplained testicular failure, it is less likely that a defect in a single gene causes infertility. The cause of testicular failure may be multifactorial, and not only genetic defects, but also environmental factors may contribute to the underlying pathology.66,75

Recently, global approaches to the examination of candidate genes have become available for the study of male infertility.76-78 Advanced techniques, such as SNP arrays, array comparative genomic hybridization analysis, and whole-genome or exome analysis through next generation sequencing, have become the popular ways to perform genetic investigation of testicular failure and will enable researchers to analyze multiple genes in parallel.79-82 There are several available platforms that can be used for genome-wide studies, each with its advantages and disadvantages in terms of resolution, genomic coverage, cost and complexity of analysis (Table 1).4 Careful data analysis of whole-genome studies performed with well-phenotyped samples is critical for successful identification of novel genetic association with infertility. In addition, considering the involvement not only of spermatogenic genes, but also of their regulatory elements are necessary to understand the complex genetic basis of male infertility.

We still have a long way to go to more completely characterize the genetic basis for testicular failure and other idiopathic cases of male infertility. Further studies must be performed to determine the exact roles of each of these genes to understand the overall mechanism of testicular failure. With the advent of these high throughput screening methods, thousands of genes can now be evaluated simultaneously. This could provide new opportunities for studying the genetic causes of testicular failure.

**Table 1: Advantages and disadvantages of the available “whole-genome” options for genetic analysis**

| Approach      | Advantages                                                  | Disadvantages                                      |
|---------------|-------------------------------------------------------------|----------------------------------------------------|
| CGH microarray| CNV information, less costly than sequencing                 | Limited resolution, no SNP/mutation information,   |
|               |                                                             | relatively complex analysis                        |
| SNP microarray| SNP/CNV information, less costly than sequencing            | Limited resolution, no point mutation information, |
|               |                                                             | relatively complex analysis                        |
| Exome sequencing| High-resolution: SNP/mutation/CNV information              | Limited to coding regions, complex analysis, more  |
|               |                                                             | costly than microarrays                            |
| Whole-genome sequencing| High-resolution: SNP/mutation/CNV information, including noncoding regions | Complex analysis, about 10X more costly than exome sequencing |

Reproduced with permission from Aston4. CNV: copy number variation; SNP: single nucleotide polymorphisms; CGH: comparative genomic hybridization
CONCLUSIONS
Testicular failure is the most difficult form of male infertility to treat, and in many cases, a genetic factor is considered to be the underlying cause. Current genetic tests for infertile men, including karyotyping and Y chromosome microdeletion testing, are widely available and are of direct benefit to infertile couples. Results of current researches suggest that accurate transmission of genetic information is essential for normal fertilization and embryo development. This is of particular importance, given the risk of transmission of genetic abnormalities to the offspring via assisted reproduction technology. Hopefully, the recent progress in the development of whole-genome-based techniques and the large-scale analysis will help to identify genetic backgrounds of male infertility and be clinically beneficial to the infertile couples.

AUTHOR CONTRIBUTIONS
SHS and RR drafted the manuscript. KC and DJL revised the manuscript. All authors have read and approved the final version of the manuscript.

COMPETING INTERESTS
The authors declare no competing interest.

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