Genetics of Male Infertility

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KEYWORDS

• Y chromosome microdeletion • Klinefelter syndrome • CBAVD • Germline mutation • Male infertility

KEY POINTS

- Much of idiopathic male infertility is likely to have a genetic cause.
- Men who have nonobstructive azoospermia or severe oligospermia with total motile count less than 5 million should have a karyotype and Y chromosome microdeletion.
- Klinefelter syndrome (47,XXY) is the most common chromosomal abnormality with a frequency of 1:600 males and has a wide spectrum of clinical presentation.
- Men with an *AZFa*, *AZFb*, *AZFb/c* microdeletion uniformly have complete absence of spermatogenesis.
- If a male has congenital bilateral absence of the vas deferens, it is critical to offer him and his partner genetic testing for cystic fibrosis mutations as well as genetic counseling.

INTRODUCTION

Approximately 1 in 6 couples in the Western world is not able to conceive spontaneously after 1 year of unprotected intercourse; in nearly half of these couples, the male partner has 1 or more semen parameters below the WHO cutoffs for normozoospermia.[1–4] Although the sequencing of the human genome in 2003 heralded a new era of genetic medicine, it will likely take decades to realize the potential of this project. Male infertility, in part due to the nature of the condition, remains largely unexplained. The cause of most cases of male infertility or subfertility remains unknown; monogenic disorders (eg, cystic fibrosis [CF], Kallman syndrome), cytogenetic abnormalities (eg, Klinefelter syndrome [KS; 47,XXY]), and Y chromosome deletions account for only up to 30% of cases.[5] The proportion of the remaining male factor cases that can be attributed to genetic causes is currently unknown, but it is likely that aberrations in many additional genes underlie a significant proportion of male infertility/subfertility because sperm production requires the coordinated action of thousands of genes, and knocking out any 1 of hundreds of genes in mice results in subfertility phenotypes in males.[6] However, discovering such genes in humans has proved challenging.[1–3,5,6]

Based on studies of animal models, however, it is likely that genetic variation that alters gene expression or function accounts for a significant proportion of male subfertility. For example, knock outs of or mutations in hundreds of genes cause subfertility phenotypes in male mice.[6] This is not surprising given that sperm development and maturation require the coordinated action of thousands of genes. However, identifying the variation and specific genes that are essential for reproductive success in humans has been extremely challenging for 2 reasons. First, because of the nature of the condition, it is virtually impossible to conduct genome-wide family-based studies of infertility, approaches that have been successful for identifying genes for many conditions with monogenic, and even some with complex genetic causes. Second, male infertility is a heterogeneous condition that can result from aberrations of many different genes. This is due in part to strong selection pressure against transmission of these genetic variants. As a result, candidate gene association studies (or even genome-wide association studies [GWAS]) of cases (infertile) and control (fertile) men would not likely be successful because only a small proportion of the cases are expected to share the same genetic abnormality.

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This is shown by the relative paucity of specific genetic variants and genes that are robustly associated with male infertility.[7–20]

Our lack of success in explaining approximately 50% to 70% of male infertility is nowhere more apparent than in our interactions with infertile men. These men want an answer to what caused their infertility. Currently, we cannot provide this in most instances. Furthermore, technological advances such as intracytoplasmic sperm injection (ICSI) and microsurgical testicular sperm extraction (microTESE) allow us to bypass the problem and bring with them another set of questions from patients that we cannot answer.[21,22] When considering ICSI, many patients want to know what are the chances they will pass on the genetic cause of their infertility to their offspring, as well as the potential for nonreproductive effects from these genes. These are questions that currently cannot completely answered completely. Although studies suggest that assisted reproductive technologies do not seem to result in a significantly higher rate of birth defects after risk factors such as maternal age are controlled for, the role of sperm quality in reproduction is just beginning to be unraveled.[23]

In 2012, Kong and colleagues[24] published a seminal paper in *Nature* showing that the de novo mutation rate for each generation is driven largely by paternal age with paternal sperm mutation rate doubling for every 16-year increase in paternal age. Increased paternal mutations from advancing age of fathers explained 30% of the increase in autism and schizophrenia over the time period of this study. The mechanism driving this is believed to be increased de novo mutations resulting from decreased fidelity of DNA replication in spermatogenesis with advancing paternal age. These mutations result in a higher mutation rate in sperm, which are then passed on to offspring and can manifest as diseases such as schizophrenia or autism.

Studies such as that of Kong and colleagues[24] and recent work by Wang and colleagues,[25] which sequenced the entire genome of individual sperm, herald a paradigm shift in our ability to develop the next generation of genetic tools to understand and possibly treat the underlying cause of male infertility. Tools such as this provide the ability to interrogate the reproductive potential of individual sperm, unfortunately, at this time, this cannot be done without destroying them. However, this technology holds incredible potential to determine the reproductive potential of an individual sperm.

Voltaire said, “with great power comes great responsibility.” In many ways, ICSI and microTESE have given us incredible power to treat male infertility. With this power, comes the ethical and moral responsibility to understand the genetic causes of male infertility for our patients and their offspring. Much of the potential of the Human Genome Project will be brought to bear on the genetic causes of male infertility.

This article examines some basic concepts that are prerequisite to any examination of the genetic causes of male infertility and reviews who should be evaluated and the current tools for genetic evaluation as well as their limitations. An overview of state of the art research in the field and what the landscape will look like in 2034 are presented.

PHENOTYPE DEFINITIONS

Studying the genetics of male infertility is complex because many of the tools of genetic analysis such as linkage mapping, family studies, and complex pedigree analysis are rendered useless by the nature of the condition. Furthermore, male infertility exists on a spectrum and is likely the result of the contribution of 100s if not 1000s of genes to a man’s overall reproductive potential.[2] To study this or any other genetic condition, accurate phenotyping is essential. To determine the precise genetic cause of male fertility, robust definitions that can clearly differentiate men into similar groups for analysis are essential. If this often overlooked but critical step cannot be completed, our efforts are doomed to failure. Although significant progress is being made in genomic, proteomic, and metabolomics biomarkers of male infertility, the limiting factor in this work is lack of accurate phenotyping of these men from a clinical and molecular standpoint (**Table 1**).[26] Another key component of accurately phenotyping men is to define accurate inclusion and exclusion criteria to establish a uniform cohort of men for analysis (**Table 2**).

Previous investigators have focused on men with nonobstructive azoospermia (NOA) to identify a pure phenotype with a uniform condition.[9,14,20] Although this approach is appealing in that NOA is certainly a reproducible end point and clearly defines a population of patients, it has not been successful in identifying genetic causal variants that explain large portions of male infertility.[7–20] Much of this is believed to be due to racial and ethnic differences in genetic carrier frequencies and the 100s of genetic defects that can result in an NOA phenotype.[5] Given that most men do not realize their full reproductive potential, that birth outcomes are also dependent on female factors, and that semen analyses are notoriously variable, NOA provides an attractive phenotypic definition for male infertility.[27] The problem with using men with NOA as a phenotypic definition of male factor infertility is that significant numbers of men with

Table 1
Summary of possible demographic data and phenotypes useful for genetic analyses of male fertility

Demographic data	Semen analysis
Age (y)	Volume (mL)
Partner's age (y)	Sperm count
Race/ethnicity	% Motility
Body mass index (kg/m^2)	Total motile count
Hormones	% Progressive motility
Follicular stimulating hormone (mIU/mL)	Average velocity
Luteinizing hormone (mIU/mL)	Mean amplitude of lateral head movement
Total and free testosterone (ng/dL)	Linearity
Clinical	Beat frequency
Months of infertility	Morphology (% normal)
Female factor present in partner	% Head defects
Anatomic	% Neck/midpiece defects
Testis longitudinal axis (cm)	% Cyoplasmic defects
Nonsevere varicocele (grade I or II)	% Tail defects

Table 2
Inclusion (A) and exclusion (B) criteria for clinical subjects in genetic studies of male infertility

A. Inclusion Criteria	
Men aged 18–65 y in a committed relationship	
No previous paternity	
B. Exclusion Criteria	
Medical history	Cryptorchidism/orchidopexy Severe testicular trauma or torsion Previous inguinal surgery Vasectomy Radical pelvic surgery Chemotherapy Pelvic external beam radiotherapy/ brachytherapy Cancer (other than nonmelanoma skin cancer) HIV/AIDS Mumps orchitis CF or CBAVD Spinal cord injury
Hormonal	Hypogonadotropic hypogonadism Hyperprolactinemia Hyper or hypothyroidism Diabetes mellitus with HbA1C >10% Exogenous steroid use
Anatomic	Grade III varicocele Severe phimosis Presence of testicular mass Buried penis due to morbid obesity
Genetic	AZF microdeletion Klinefelter syndrome Intersex disorder CFTR mutation
Semen analysis	Seminal hypovolemia (volume<1.5 mL)
Sexual history	Ejaculatory dysfunction

Abbreviations: CBAVD, congenital bilateral absence of the vas deferens; CFTR, cystic fibrosis transmembrane conductance regulator; HIV, human immunodeficiency virus.

causal genetic variants that contribute to subfertility or severe oligozoospermia through genetic pathways distinct from those that cause NOA may be missed. Furthermore, because NOA is only a small subgroup of men with male infertility, it is unclear if understanding the genetic causes of NOA will translate directly into deciphering other aspects of male infertility.

Alternative phenotypic definitions for male infertility have their own problems and limitations as well. Specifically, using patient self-reports of their fertility is problematic and, if used, needs to be done in a validated and controlled manner; it will only work in specific populations where men realize their true genetic reproductive potential. Many couples now seek assisted reproductive technologies before attempting to conceive for 12 months.[1,28] Alternatively, investigators have relied on semen analyses to define groups of men with oligozoospermia, but variability in semen analyses mandates use of multiple semen samples to define these groups of men.[27,29] Case-control definitions are also problematic because semen analyses parameters, such as total motile counts, are quantitative continuously distributed traits that show large intraindividual and interindividual variation. Thus, dichotomizing total motile count would fail to detect an overall reduction in sperm count caused by a genetic factor, unless the cutoff point for the case definition was set very low.[2] Finding accurate controls for these studies has also proved to be problematic.

One alternative to just relying on NOA or oligozoospermia on a semen analysis is to define more

robust clinical phenotypes that tie in other relevant pieces of clinical information such as the physical examination and hormone levels (Table 3). Male and female reproductive hormone levels are an integral part of an infertility evaluation and frequently change clinical management.[30] For men, the level of follicular stimulating hormone (FSH) is often more predictive of spermatogenesis capacity than a semen analysis, assuming absence of azoospermia, and luteinizing hormone (LH) and testosterone (T) often identify treatable hypoandrogenism.[30] Multiple studies have demonstrated that levels of FSH, LH, T, and anti-Mullerian hormone (AMH) in men have significant heritability (56%–90%).[31,32] Previous studies of twins have suggested that levels of these hormones are heritable,[31,32] but further genetic studies on these important biomarkers of reproductive health are lacking.

GENETICS BACKGROUND

Basic Genetics

The central dogma of biology dictates that DNA makes RNA, which makes protein. Proteins form the building blocks of life. The basic building blocks of genes, the genetic code, consists of 4 deoxyribonucleotides (adenylic acid [A], guanylic acid [G], thymidylic acid [T], and cytidylic acid [C]). Two strands of DNA are joined together to form a double helix with A binding to T and G binding to C. DNA consists of introns, sections of DNA that do not code for proteins and exons, and sections of DNA that code for proteins. Although only a small fraction of DNA codes for proteins, recent insights from the ENCODE study have revealed that the intervening DNA is not random noise but serves to regulate the exons or coding segments.[28,33] The DNA of each gene is transcribed to make mRNA. During translation, each 3-unit nucleotide codon is translated by the ribosome to make a specific amino acid. Sequences of amino acids then make specific proteins, the functional end product of each gene.

DNA is tightly packaged in the nucleus of cells. It is set in a background of histone proteins, stacked and compacted to form each of the 46 chromatids, which consist of a short (p) arm and a long (q) arm. One of the chromatids is of paternal origin and 1 is of maternal origin. These chromatids make up the diploid genome which consists of 22 pairs or autosomes numbered from largest to smallest and 1 pair of sex chromosomes (X/Y or XX) (Fig. 1).[34]

DNA is replicated in the process of mitosis and meiosis. Mitosis occurs in all cells and precisely replicates the DNA to produce 2 genetically identical diploid daughter cells from each mother cell. Meiosis occurs only in germ cells and involves a process of recombination and reduction in chromosome number to a haploid spermatozoa or oocyte. Fusion of an oocyte and a spermatozoa result in restoration of the diploid number of chromatids.

DNA mutations

A mutation is an alteration in DNA that can be passed from parent to daughter cells. There is a critical distinction between somatic mutations and germline mutations. Somatic mutations are passed from mother to daughter cells, but not passed on to the next generation. The rate of de novo germline mutations is not insignificant and tends to increase as people age.[24,35] Both germline and somatic mutations may result in a change in the amino acid sequence of a protein or the length of genes (insertions or deletions). In this article, the discussion of genetic inheritance focuses on germline genetic disorders and this model of inheritance.

DNA polymorphisms

Polymorphisms are alterations in the DNA found in at least 1% of the population. Generally speaking, DNA polymorphisms do not cause disease but may alter the risk or severity of disease.[36] There are several types of polymorphisms. The most common and most relevant for male infertility are single nucleotide polymorphisms (SNPs), which occur in up to 1 in 100 base pairs for some genes and typically do not cause disease. SNPs are specific areas of DNA that vary between individuals in a population. An allele is a specific variant of DNA at a specific location, whereas a genotype is the alleles an individual received from each parent at a given genomic position such as A/C. A haplotype is the alleles that were each received together from 1 parent.[5] GWAS attempt to determine whether the genotypes of certain SNPs are associated with complex diseases. These studies generate massive amounts of data and are complex to interpret but are statistically relatively straightforward although computationally intensive; they rely on millions of *t* tests to examine the association of the genetic predictor (SNP genotypes) with the outcome of interest. Examination of millions of SNPs in a given study often results in stringent criteria for genome-wide significance ($P<1 \times 10^{-8}$) after correcting for multiple comparisons (Fig. 2).[5] GWAS, when properly performed, adequately powered and correctly interpreted, may have the power to yield insight into complex diseases such as male infertility.

Genetic Disorders

Genetic disorders can be divided into single gene disorders or mendelian disorders, chromosomal disorders, and nonmendelian genetic disorders.

Table 3
Summary of male infertility phenotype components

Male Infertility Metric	Rationale for Measurement
Demographic	
Age (y)	Semen quality decreases with age >25 y[24,122–124]
Race/ethnicity	Significant racial variability in male infertility prevalence,[124,125] care seeking behavior,[126] and semen analysis profiles mandates adjustment by race[127]
Body mass index (BMI) (kg/m^2)	Increasing BMI is associated with declining semen quality[128]
Partner age (y)	Increasing female age is associated with decreased fertility
Hormones	
Follicular stimulating hormone (FSH) (mIU/mL)	FSH correlates directly with spermatogenesis potential and is significantly less variable and more heritable than the parameters of a semen analysis[30,32,129]
Luteinizing hormone (mIU/mL)	Indicates adequacy of Leydig cell function to maintain adequate testosterone levels for spermatogenesis[129]
Free testosterone (ng/dL)	Adequate free testosterone is necessary to optimize human spermatogenesis and does not always correlate with total testosterone[130,131]
Total testosterone (ng/dL)	Total testosterone <280 ng/dL has sensitivity of 91.0% and specificity of 73.7% for low free testosterone[132]
Semen Analysis	
Volume (mL)	Seminal hypovolemia (<1.5 mL) indicates obstructive azoospermia or retrograde ejaculation, not spermatogenic failure[28]
Sperm count (millions of sperm)	Pregnancy rates decline with decreasing sperm counts[124,127]
Total motile count (TMC) (millions of sperm)	Clinically, TMC is used to determine the severity of male factor infertility and to guide clinical treatment. Pregnancy rates are believed to decline linearly with reduced TMC <15 million[28]
% Motility	Decreases in motility can indicate genetic defects in spermatogenesis that can result in populations of immotile sperm[28]
% Normal morphology by Kruger strict criteria	Previous work has found that genes associated with reduced reproductive potential are also associated with specific morphologic defects.[133,134] Decreased % of normal forms may be associated with decreased fertility and is directly related to the quality of the germinal epithelium[28]
% Head defects	Previous work has found that genes associated with reduced reproductive fitness are also associated with sperm head defects[134]
Anatomic	
Testis longitudinal axis	80% of testicular volume is composed of the seminiferous tubules, where spermatogenesis occurs. Thus, testicular size is directly proportional to reproductive fitness and does not have the variability seen in semen analyses[28,129]
Presence/side/grade of varicocele	Varicoceles are associated with oligoasthenoteratospermia and are found in up to 50% of men presenting to infertility clinics. However, they are often an incidental finding and are typically not causative of severe defects in spermatogenesis. Thus, men with severe (grade III) varicoceles are excluded because the genetic factors causing varicoceles are unknown but are believed to differ from those causing defects in spermatogenesis[28]
Clinical*	
Months of infertility at time of evaluation	Reproduction is an inefficient process, even in fertile couples, with chances of fertilization approaching 20% under ideal conditions. Severity of male factor infertility correlates linearly with length of time to natural conception without assisted reproductive technologies.[135] Infertility is defined clinically as the lack of pregnancy after 1 y of attempts at pregnancy[28,124,127]

(*continued on next page*)

Table 3
(*continued*)

Male Infertility Metric	Rationale for Measurement
Months until natural conception	See above
Months until conception with intrauterine insemination	Chances of pregnancy with intrauterine insemination are roughly 15%–20% per cycle.[28] Because intrauterine insemination success usually requires a TMC >5 million and is proportional to sperm function, this can be used as a surrogate for reproductive fitness

All the examples mentioned briefly here are discussed in detail in the following sections. Single gene or mendelian disorders are caused by a mutant allele or pair of alleles at a single genetic locus. These mutant alleles may either be inherited from parents or occur de novo in spermatozoa or oocytes. Regardless of where they come from, once present, all these mendelian disorders are passed on to offspring in 1 of several standard modes of inheritance. Autosomal dominant mutations are expressed with the inheritance of a single mutant allele, whereas autosomal recessive mutations require the disease-causing mutation to be present on both alleles of a gene. CF is a classic example of an autosomal recessive mutation.[37] X-linked disorders cause disease in men (46,XY) with the mutation and in women who inherit 2 copies of the X-linked mutation. Thus, these diseases affect men more than women. Kallman syndrome is an example of an X-linked disorder.[1]

Chromosomal disorders are caused by the loss, gain, or abnormal arrangement of 1 or more of the 46 chromosomes.[1] Although most chromosomal disorders are de novo events that result from significant mutations in the parent germ cells, they often demonstrate a modified pattern of mendelian inheritance. These disorders can be classified as either numerical/structural or microscopic/submicroscopic. There are 2 categories of numerical chromosomal abnormalities: (1) polyploidy, a chromosomal number that is a multiple of 23 in which there are extra copies of all chromosomes; (2) aneuploidy, a gain or loss of 1 or more chromosomes. Aneuploidy is typically denoted as the number of extra or missing copies and the chromosome; for example, trisomy 21. Aneuploidy is significantly more common than polyploidy. Mosaicism results when individuals have tissues consisting of a mixture of cell lineages with different chromosomal complements. A classic example of a numerical aneuploid chromosomal disorder where mosaicism is common is KS with a karyotype of 47,XXY.[38]

Microscopic or submicroscopic chromosomal disorders result from a loss, gain, or rearrangement of material within a chromosome or between

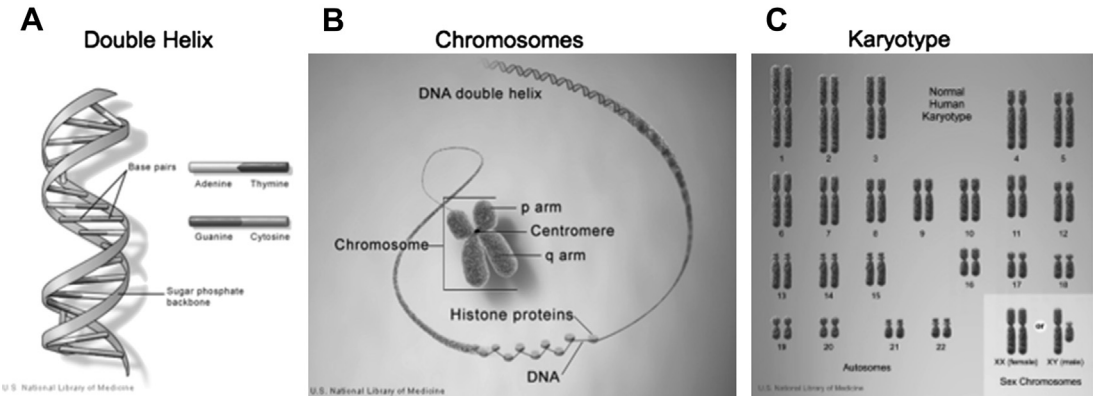

Fig. 1. DNA structure. (*A*) DNA is arranged in a ladder twisting in the form of a double helix. The base pairs adenosine-thymine and guanine-cytosine form the rungs of the ladder. A sugar phosphate backbone forms the supports of the ladder or helix. (*B*) DNA strands are spooled and then condensed into fibers that are further compacted by looping around histones to form a chromosome that consists of 2 chromatids joined by a centromere. Each chromatid has a p arm (*short*) and a q arm (*long*). (*C*) The 22 autosomal chromosomes and the 1 sex chromosome can be laid out in a karyotype to determine if there are any grossly visible chromosomal abnormalities. (*Courtesy of* The National Human Genome Research Institute, National Institute of Health, Bethesda, MD.)

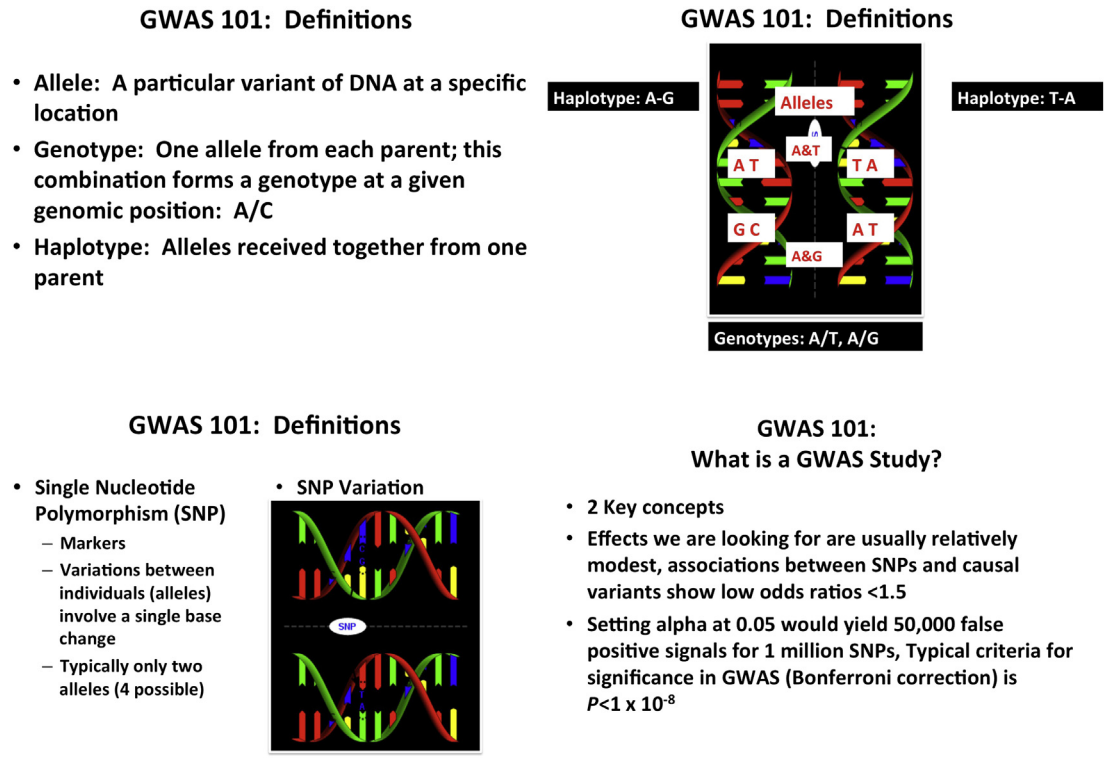

Fig. 2. GWAS description. Alleles are variants of DNA at a specific location. The combination of alleles inherited from each parent form a genotype at a given genomic position. A haplotype denotes alleles that were all received together from 1 parent. The key concept here is that most adult cells are diploid (2n) and have 2 copies of the DNA, 1 from each parent. Thus, when a specific location or allele is examined, an individual's genotype is composed of the basepairs at each of their 2 copies of genetic material. SNPs are variations of alleles between individuals that involve a single base change. GWAS studies use SNPs as the predictors and disease states as the outcomes. As the effects being looked for are very modest and tests are repeated millions of times, large sample sizes are often required to reach statistical significance ($P<1 \times 10^{-8}$).

chromosome. The key distinction between these and numerical chromosomal abnormalities is that only a piece of the chromosome is affected, not the entire chromosome. A common mechanism for these disorders is reconfiguration of blocks of DNA or low copy repeats, which are 10 to 400 kb long, have nearly identical sequences, and are dispersed throughout the chromosome accounting for 5% of the human genome.[34,39,40] A classic example of this is deletions of part of the Y chromosome resulting in microdeletions leading to male infertility, the so-called azoospermia factor (AZF) disorders.[40]

Nonmendelian disorders account for most human disease. Study of these diseases is significantly more complex than for mendelian disorders.[41] GWAS have been the mainstay used to investigate complex, polygenic, nonmendelian diseases (see Fig. 2).[42] Other inheritance patterns exist such as expansion of trinucleotide repeats, mitochondrial inheritance, genomic imprinting, and uniparental disomy but these are beyond the scope of this article. Studying spermatogenesis is complex and requires understanding how 1000s of genes operate together and the development of new tools to examine complex nonmendelian traits such as GWAS.

CURRENT GENETIC TOOLS

Spermatogenesis involves the coordinated action of 1000s of genes.[2,5,43] Although any number of these could make excellent targets for diagnostic tests of male factor infertility or subfertility, only a small handful of genetic variants have been clearly linked to spermatogenic failure in a robust and reproducible manner.[26]

Congenital Bilateral Absence of the Vas Deferens

Congenital bilateral absence of the vas deferens (CBAVD) occurs in approximately 1% of infertile men and is diagnosed on physical examination, prompting subsequent genetic testing.[44] Men with

this condition present with obstructive azoospermia, absence of the vas deferens, and possibly absence of the distal part of the epididymis, hypoplastic seminal vesicles, and consequent seminal hypovolemia (<1 mL) and an acidic ejaculate (pH 6.5–7.0).[45] CBAVD is found in all patients with clinical CF and CBAVD without other clinical manifestations of CF is believed to result in people with mutations that confer at least some functional forms of the gene that causes CF when it is completely absent.[46]

CF affects 1:1600 people of northern European descent and genetic testing must account for ethnicity to identify the 850 or so genetic variants known to cause CF.[47,48] Obstructive pulmonary disease caused by thickened epithelial secretions is the defining feature of clinical CF; pancreatic exocrine failure from the same mechanism is also common.[49] Absence of the vas deferens occurs in all males with clinical CF.[50] Clinical CF requires inheritance of maternal and paternal CF genes. The CF gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR), a protein crucial for the maintenance of viscosity through optimal sodium and chloride balance in epithelial secretions. If only 1 copy of an abnormal CFTR gene is present along with another normal copy, the patient is a carrier and pancreatic and respiratory function are unaffected. The severity of the phenotypic picture of CF, from carrier to clinical CF, depends on the functionality of the copies of the CFTR genes that individuals inherit from their parents. The least severe form of CF is CBAVD, where the CFTR protein allows for adequate pancreatic and respiratory function but results in vasal agenesis.[46] Although the vas, epididymis, and seminal vesicles are of mesonephric origin, they become atretic in the later stages of development, indicating that the mesopnephric ducts are embryologically normal and men with CBAVD have normal renal units.

Although more than 1500 mutations can cause CF and CBAVD, a 3 base pair deletion, deltaF508, is the most common mutation found in northern Europeans with CF and CBAVD.[51] deltaF508 is a severe mutation and the homozygous state results in clinical CF. In men with CBAVD, complete genome sequencing results in detection of 90% of abnormal CFTR alleles (the other 10% are presumed but not detectable); 88% carry a severe mutation (absent CFTR function) in combination with an allelic mild mutation that preserves some CFTR function.[50,52] The most frequent mutation detected is deltaF508 (24%) and the second most common is IVS8-T5 (17%). T5 causes mild CFTR malfunction and is present in up to 5% of the general population. The most frequent genetic combination in patients with CBAVD was deltaF508 in trans to IVS8-T5 (16.5%). Most other CFTR mutations were at a frequency of 3% or less.[50,53] Unilateral absence of the vas deferens should also be evaluated with renal ultrasonography because many of these men have renal agenesis or ectopia.[54] Another variant of this is congenital nonunion of the vas deferens with the epididymis, which is poorly understood and may lend itself to microsurgical reconstruction in some instances.

If no mutations in CFTR are discovered in the male, another possible cause of CBAVD is from abnormal differentiation of the mesonephric ducts before week 7 resulting in unilateral renal agenesis or ectopy and CBAVD. This scenario occurs in its severe form as Potter syndrome, is unrelated to CF, has an unknown genetic basis, and warrants renal ultrasonography in men with CBAVD to identify this entity.[55]

Perhaps the most critical portion of an evaluation of CBAVD is workup of the female partner for CFTR mutations. According to the American Urological Association (AUA) Best Practice Policy Committee's Report from 2010 on the *Evaluation of the Azoospermic Male*, "Testing for cystic fibrosis transmembrane conductance regulator abnormalities should include at minimum a panel of common point mutations and the 5T allele. Gene sequencing may be considered in couples where the wife is a carrier and the husband with congenital bilateral absence of the vasa deferentia tests negative on a routine panel of cystic fibrosis transmembrane conductance regulator mutations."[56] (https://www.auanet.org/common/pdf/education/clinical-guidance/Male-Infertility-b.pdf). Referral to a genetic counselor is a critical component of this process.

Men with CBAVD and CF should undergo genetic screening and are then candidates for microsurgical or percutaneous sperm aspiration procedures or testicular sperm extraction for use in conjunction with ICSI.[57] Preimplantation genetic screening should be done if the patient's partner harbors a CFTR mutation, resulting in a 25% chance of offspring inheriting abnormal alleles from both parents and developing clinical CF.[58] The key points of the evaluation of a man presenting with CBAVD are summarized in **Table 4**.

Karyotype Abnormalities

Karyotype

Numerical and structural chromosomal abnormalities are 8 to 10 times more prevalent in infertile men than in fertile controls (3% in oligospermia and 19% in NOA).[38,59–61] Obtaining a karyotype in infertile men is recommended after a careful

Table 4
Key points for evaluation of men with CBAVD

	Diagnosis	Workup	Treatment
Critical point	Absence of vas deferens on examination?	CFTR mutation testing for man and wife	Sperm extraction and ICSI with fresh/frozen sperm Preimplantation diagnosis for men with CBAVD where both husband and wife are carriers of severe CFTR mutations (eg, deltaF508)
Ancillary points	Seminal hypovolemia?	Consider gene sequencing if no mutations detected in man and wife is a carrier	Referral to a genetic counselor, testing of siblings
	Family history of CF?	Renal ultrasonography for man if no detectable mutations	Cost considerations for patients

discussion of the risks and benefits and cost of genetic testing with the patient. A karyotype typically costs $400 to $900 and is rarely covered by insurance. The AUA guidelines recommend a karyotype in all men with NOA and a total motile count less than 5 million.[56]

47,XXY KS

KS (47,XXY) is the most common chromosomal abnormality with a prevalence of 1:600 in males and is the most common genetic cause of azoospermia.[38] KS has a wide clinical spectrum but all males have atrophic testes (8–10 cm^3) and marked increase in FSH and LH levels. In addition, approximately 10% to 20% of men with KS are mosaic with cells demonstrating 47,XXY and 46,XY karyotypes or other mosaic compositions.[62] Spermatogenesis is typically severely limited in all men with nonmosaic KS, and most have azoospermia. However, up to 8.4% of men with nonmosaic KS do have sperm in their ejaculate. FSH is increased in response to abnormal spermatogenesis. Regardless of testosterone levels, LH is typically increased as a result of maximal stimulation of Leydig cells that produce androgen inefficiently.[62–65]

The presence of an additional X chromosome results in not only spermatogenic and androgenic failure but also gynecomastia, expressive language difficulties, higher mortality from breast cancer and non-Hodgkin lymphoma (standardized incident ratios of 57.8 and 3.5, respectively), a decreased risk of prostate cancer, and a higher incidence of extragonadal germ cell tumors mandating karyotyping in men presenting with these tumors.[38,65–68]

Men with KS can present in a myriad of ways. If they do not have adequate androgenic potential, they typically present to a pediatric endocrinologist with delayed or absent virilization at the time of puberty.[64] Others are referred in adolescence because of small testes and many are discovered only at the time of infertility evaluation.[61] Men with KS typically have normal libido and erectile function.[60]

Research among fathers of offspring with KS has demonstrated that the frequency of XY sperm increases significantly with paternal age.[69] Some have also argued that spermatogenic potential decreases with advancing age in men with KS and many have raised concern about high rates of aneuploidy sperm among men with KS.[70–72] Despite this concern, more than 100 births have been described in the literature with no aneuploid offspring.[73–77] Part of the debate on this issue stems from the lack of consensus on the exact mechanism of 47,XXY men producing 23,X or 23,Y sperm. Two hypotheses have been proposed to explain this. Either the 47,XXY spermatogonia have the potential to complete meiosis resulting in both aneuploid and haploid sperm or the testicular environment hypothesis, whereby spermatozoa of men with 47,XXY KS arise from patches of 46,XY spermatogonial stem cells in the testis and increased aneuploidy rates are from an aberrant testicular environment.[78]

MicroTESE has been demonstrated to yield successful sperm retrieval in up to 69% of men with KS.[79,80] No characteristic or algorithm has been shown to successfully predict the presence of sperm in azoospermic men with KS.

46,XX male syndrome

46,XX testicular disorder of sex development (46,XX male syndrome) is found in 1:20,000 male births and is a rare genetic cause of infertility in

phenotypic males.[81] The sex-determining region (SRY) is the key genetic component, normally residing on the Y chromosome, that results in testicular development, testosterone production, and a male phenotype.[81] Ninety percent of these 46,XX men have SRY, which normally resides on the distal portion of Yp, translocated to the X chromosome or an autosome.[82] The small number of these 46,XX SRY-negative men are believed to have undefined genetic abnormalities permitting gonadal differentiation.[83] 46,XX men are phenotypic males but have smaller testes, decreased height, and are uniformly infertile because of the absence of other genetic factors found on the Y chromosome that are critical for normal spermatogenesis, such as the azoospermia factors (*AZFa*, *AZFb*, *AZFc*).[84] Thus, the karyotype is prognostic in these patients and the patient is not a candidate for microTESE or testis biopsy.

Other karyotype abnormalities

Infertile men can have other Y chromosome abnormalities including mosaicism, ring Y, truncated Y, and isodicentric Y.[85–87] Ring Y chromosomes are formed by loss of genetic material and circularization of the remaining Y chromosome. Patients with ring Y chromosomes should undergo AZF microdeletion assays to determine if these regions are present.[87] Referral to a genetic counselor is crucial in the evaluation of these patients. Robertsonian and reciprocal translocations are found more commonly in the oligospermic than the azoospermic population.[88] The key features of kayotypic abnormalities are summarized in **Table 5**.

Y Chromosome Microdeletions

Ninety-five percent of the Y chromosome is contained in the male-specific region of the Y chromosome or MSY and contains unique genetic material for sex-specific embryogenesis such as the SRY. However, the Y chromosome does not recombine in the same manner as autosomal chromosomes; it does contain approximately 8 massive palindromic sequences that enable maintenance of the fidelity of the genetic material on the Y through intrapalindrome homologous arm-to-arm recombination.[86,89] From an evolutionary standpoint, the Y chromosome is highly efficient, containing the entire male phenotypic developmental pathway in a minimum of DNA.[90] However, unlike its autosomal counterparts, it does not have the luxury of having 2 copies of critical genetic material and the loss of any of its material has reproductive consequences for men.[91]

Any deviation from the intrapalindromic arm-to-arm recombination can lead to ectopic homologous recombination.[92] Errors occur when 2 spatially separated palindromic segments of the Y chromosome are erroneously combined, deleting all the intervening genetic material. These losses are referred to as microdeletions because they are not visible on standard karyotype analysis.

Loss of portions of the Y chromosome are detected in roughly 10% of men with NOA and 5% of men with severe oligospermia, but significant ethnic variations in these rates and the types of deletions exist.[40,93–95] Microdeletions are most common on the long arm of chromosome Y, Yq, and deletions in this are related to spermatogenic

Table 5
Key points for karyotype analysis

	Diagnosis	Workup	Treatment
Klinefelter Syndrome 47,XXY			
Critical point	Most common chromosomal abnormality (1:600)	Karyotype, FSH/LH, total testosterone, albumin, SHBG, estradiol	MicroTESE if azoospermic Cryopreservation of sperm if severely oligospermic
Ancillary points	Wide clinical spectrum 10%–20% mosaic Increased risk of breast cancer, non-Hodgkin lymphoma	—	—
46,XX Male Syndrome			
Critical point	Rare (1;20,000), phenotypic men	Karyotype, FSH/LH, total testosterone, albumin, SHBG, estradiol	Donor sperm
Ancillary points	Smaller testes, decreased height	—	—

Abbreviation: SHBG, sex hormone binding globulin.

failure. The AZF region contains key genes for sperm development and has 3 subgroups: *AZFa*, *AZFb*, *AZFc* (**Fig. 3**). Multiple deletions in the *AZFc* areas are the most common, occurring in up to 10% of men with NOA and 1:4000 men overall.[96–98] *AZF b/c* microdeletions are those in which the recombination boundaries encompass both the *AZFb* and *AZFc* regions.[92] Nearly all AZF microdeletions occur de novo but, once present, they are passed on to all male offspring of an effected man making genetic counseling critical.[99]

All the AZF microdeletions have no phenotypic or health consequences other than their effect on spermatogenesis. The *AZFa*, *AZFb* and *AZFb/c* microdeletions remove critical genes for spermatogenesis and men with these microdeletions do not have sperm. However, with the *AZFc* microdeletion, sperm is found in 70% of men on microTESE.[84] A Y chromosome microdeletion assay is readily available as a blood test that can detect AZF microdeletions and should be obtained in all men with NOA or severe oligospermia (total motile count less than 5 million) before any attempts at sperm retrieval.

The 2 main genes critical for spermatogenesis and located in *AZFa* are *USP9Y* and *DBY* or *DDX3Y*. Deletion of both these genes results in Sertoli cell only syndrome and complete absence of sperm on microTESE.[98,100] Deletions in the *AZFb* region causes arrest of spermatogenesis at the primary spermatocyte stage.[101] The main genes in this region are *RBMY1*, which codes for a testis-specific splicing factor, and *PRY*, which is involved in apoptosis.[102,103]

AZFc microdeletions are not as easily characterized as they range from smaller subdeletions to intrachromosomal recombinations and even complete deletions.[104] Although spermatogenesis can still occur in the presence of an *AZFc* microdeletion, it is markedly reduced and these patients are typically azoospermic. Study of *AZFc* microdeletions is further complicated by significant ethnic variability depending on the genetic makeup of the haplogroups examined. One of the most frequent subdeletions of the *AZFc* region is the gr/gr subdeletion, which removes half the *AZFc* content, but ethnic variability among haplotypes has made the study of this subdeletion difficult.[105,106] Like the other AZF regions, AZFc also contains genes involved in spermatogenesis, *DAZ*. The *DAZ* genes are expressed in all stages of spermatogenic development.[107,108]

A blood-based assay of the AZF microdeletions can yield critical prognostic information before attempted sperm retrieval, as only men with AZFc have the potential for a successful outcome.[100] **Table 6** provides a summary of the key points for AZF microdeletions.

Other genes on the Y chromosome that are believed to play a role in spermatogenesis are *CDY*, which regulates DNA transcription through acetylation of histones, and *TSPY*, regulates the timing of spermatogenesis by signaling spermatogonia to enter meiosis.[101] Although the exact roles of these genes in male infertility remain undefined, a study of copy number variants or copies of the *TSPY* gene found that infertile patients had more copies of the *TSPY*.[109]

Hormone Levels and Epigenetics

Hormone levels

Male and reproductive hormone levels are an integral part of an infertility evaluation and frequently change clinical management.[30] Multiple studies have demonstrated that levels of FSH, LH, T, and AMH in men have significant heritability (56%–90%), but further genetic studies on these important biomarkers of reproductive health are lacking.[31,32] The sex hormone–binding globulin (*SHBG*) gene, located on chromosome 17, has been identified as having a possible role in spermatogenesis with shorter *SHBG* alleles being associated with higher sperm concentrations, but this study has not been replicated.[4] Likewise, studies

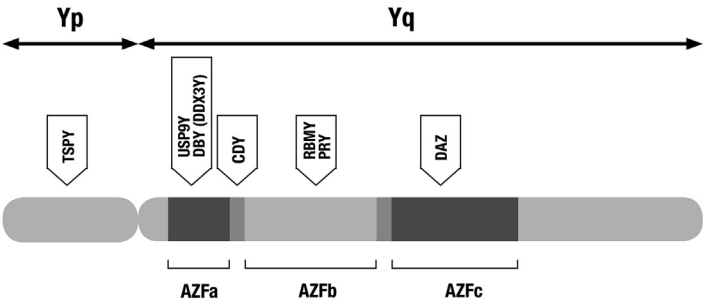

Fig. 3. AZF regions of the Y chromosome. Presence of the AZFa, AZFb and AZFc regions on the long arm of the Y chromosome with genes relevant to spermatogenesis highlighted above. The numerous palindromic sequences within the Y chromosome may combine in a myriad of ways and AZFa, AZFb, and AZFc are simply groups of these aberrant recombinations that remove specific genes. TSPY is a gene on the short arm of chromosome Y that is involved in spermatogenesis but is not part of the genes in the AZF regions. (*From* O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril 2010;93:3; with permission.)

Table 6
Key points for AZF microdeletions

	Basics	Genes Affected	Prognosis
AZFa	Rare	*USP9Y DBY or DDX3Y*	All will have Sertoli cell only on testis biopsy, no sperm
AZFb	Rare	*RBMY1 PRY*	All will have maturational arrest, no sperm
AZFc	10% of men with NOA, 1:4000 overall	DAZ	70% chance of sperm on microTESE, rarely sperm in ejaculate

of FSH receptor polymorphisms, androgen receptor gene CAG repeats, and estrogen-related genes have not yet translated into clinical assays that can assess the complex interplay between the male endocrine axis and spermatogenesis.[110–113] Regardless, refined phenotyping of cohorts of infertile men as well as larger-scale studies will hopefully allow some of these polymorphisms to be linked with clinical outcomes of infertile men.

Epigenetics

Epigenetics is defined as alterations of the genetic code that do not alter the basic DNA sequence. An example would be imprinting, the addition of a methyl group to DNA, which changes the regulation of transcription and, consequently, gene expression.[114] Seminal work by Hammoud and colleagues,[115] published in *Nature* in 2009, demonstrated that developmental promoters are extensively hypomethylated in sperm and acquire methylation during differentiation. Thus, epigenetics plays a critical role in enabling sperm to facilitate early embryogenesis. Subsequent work by this group has associated spermatic DNA methylation changes in imprinted genes with male factor infertility.[116,117] Although the exact causal relationship of this association has not been fully elucidated, and conflicting results on the exact role of methylation remain in the literature, further investigation of this area holds significant promise for unraveling the genetic underpinnings of male infertility.

GENETICS OF MALE INFERTILITY IN 2034

The state of research in male infertility in 2013 can be summarized succinctly by noting that the number of potential targets identified by GWAS, microarray studies, proteomics, metabolomics, genomics, and large cohort and case-control studies have failed to translate into any new tangible clinical assays that can classify, prognosticate, or treat male infertility.[26,118] As previously discussed, much of this is due to the inability to use the tools of classic genetics, such as pedigree studies, to examine male infertility, and the heterogeneous nature of the condition. This situation is further complicated by the lack of reliable animal models for spermatogenesis, inability to grow these cells reliably in culture, and incomplete phenotyping of most cohorts of infertile men.[2] Currently, most cases of male infertility are treated medically or surgically; bypassing the genetic problem, rather then identifying and treating the underlying issue. Although there have been tremendous research efforts in this area, the results have not yet translated into clinical practice.

Several key developments will shape the diagnostic and potentially therapeutic genetics of male infertility in 2034. First, as the costs of genetic tests continue to decrease exponentially, whole-genome sequencing, which is currently $3000 to $10,000 per sample, will decrease to a price whereby it can be routinely used in clinical practice.[5] For point of comparison, the first human genome sequenced cost $2.7 billion and took 13 years to complete (http://www.genome.gov). Second, the limiting factor in most genetic analyses is no longer the cost but the technical knowledge, computational power and biological training necessary to interpret the massive amount of data generated. Following Moore's law, as the price of computers continues to decrease and their computational power increases, this bottleneck will continue to be less of a limiting factor. Application of machine learning to genetic data is being embraced by startup companies such as Ayasdi (www.ayasdi.com) and veteran computer companies such as IBM (http://www-03.ibm.com/innovation/us/watson/). Machine learning promises to yield new insights into genetic data. Third, recent work by groups such as ENCODE have started to demonstrate that most noncoding DNA is not junk but serves to orchestrate a complex interplay of transcription factors that regulate transcription.[119–121] The type of work published by the ENCODE team is at the heart of the epigenetic changes that have recently been shown to be crucial for the role of the sperm in early embryogenesis. Fourth, the male infertility community is beginning to lay the groundwork to build andrologic equivalents of SEER (Surveillance, Epidemiology

and End Results), the large cancer database that can be linked to robust tissue specimen, phenotype, and genotype information for research.

By 2034, the decreased cost of genetic testing, improved computational infrastructure, better understanding of transcriptional regulation, and large databases will allow for several diagnostic and potentially therapeutic genetic modalities related to infertility. By studying large populations of men who are fertile, infertile, and subfertile, genetic markers can be identified that are markers of fertility and harbingers of infertility. A semen analysis will remain a key component of a male fertility evaluation but a blood test performed in tandem with the semen analysis will be able to quickly scan the 1000 key genes involved in spermatogenesis and the male endocrine axis and identify a man's reproductive potential by providing him with an assessment of his innate genetic reproductive potential. Should a man be azoospermic, this assay would also provide information about the chances of finding sperm on testis biopsy or microTESE.

Ideally, this evaluation will return a man's chance of conception per month with his partner based on a similar assay of some of the key genes involved in female reproduction. These tests will also identify groups of men who will and will not respond to medical endocrine treatments and their ideal dosing through pharmacogenomics. Although it is possible that some of the identified genes may ultimately be targets for gene therapy, it is unlikely that this treatment modality will be widespread in 2034 because of the complexity of delivery of these agents and the necessity of determining the potential reproductive consequences of their use.

SUMMARY

Statistical genetics holds incredible promise in the study of male infertility. Although significant strides have been made in understanding the genetics of male infertility, most of it remains unknown. Currently, our ability to treat male infertility has far outstripped our capability to understand its root causes. In the future, the benefits of the ongoing revolutions in genetic sequencing and the processing of this information will likely be realized so that patients can be provided with answers about the nature of their condition as opposed to just performing surgery to treat it.

REFERENCES

1. Massart A, Lissens W, Tournaye H, et al. Genetic causes of spermatogenic failure. Asian J Androl 2012;14(1):40–8.
2. Visser L, Repping S. Unravelling the genetics of spermatogenic failure. Reproduction 2010;139(2):303–7.
3. de Kretser DM. Male infertility. Lancet 1997;349(9054):787–90.
4. Lazaros L, Xita N, Kaponis A, et al. Evidence for association of sex hormone-binding globulin and androgen receptor genes with semen quality. Andrologia 2008;40(3):186–91.
5. Aston KI, Conrad DF. A review of genome-wide approaches to study the genetic basis for spermatogenic defects. Methods Mol Biol 2013;927:397–410.
6. Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. Nat Med 2008;14(11):1197–213.
7. Irie S, Tsujimura A, Miyagawa Y, et al. Single-nucleotide polymorphisms of the PRDM9 (MEISETZ) gene in patients with nonobstructive azoospermia. J Androl 2009;30(4):426–31.
8. Miyamoto T, Koh E, Sakugawa N, et al. Two single nucleotide polymorphisms in PRDM9 (MEISETZ) gene may be a genetic risk factor for Japanese patients with azoospermia by meiotic arrest. J Assist Reprod Genet 2008;25(11–12):553–7.
9. O'Bryan MK, Grealy A, Stahl PJ, et al. Genetic variants in the ETV5 gene in fertile and infertile men with nonobstructive azoospermia associated with Sertoli cell-only syndrome. Fertil Steril 2012;98(4):827–35.e1–3.
10. Bashamboo A, Ferraz-de-Souza B, Lourenco D, et al. Human male infertility associated with mutations in NR5A1 encoding steroidogenic factor 1. Am J Hum Genet 2010;87(4):505–12.
11. Harbuz R, Zouari R, Pierre V, et al. A recurrent deletion of DPY19L2 causes infertility in man by blocking sperm head elongation and acrosome formation. Am J Hum Genet 2011;88(3):351–61.
12. Hu Y, Wen W, Yu JG, et al. Genetic association of UBE2B variants with susceptibility to male infertility in a Northeast Chinese population. Genet Mol Res 2012;11(4):4226–34.
13. Hu Z, Xia Y, Guo X, et al. A genome-wide association study in Chinese men identifies three risk loci for non-obstructive azoospermia. Nat Genet 2012;44(2):183–6.
14. Zhao H, Xu J, Zhang H, et al. A genome-wide association study reveals that variants within the HLA region are associated with risk for nonobstructive azoospermia. Am J Hum Genet 2012;90(5):900–6.
15. Ji G, Gu A, Wang Y, et al. Genetic variants in antioxidant genes are associated with sperm DNA damage and risk of male infertility in a Chinese population. Free Radic Biol Med 2012;52(4):775–80.
16. Ji G, Long Y, Zhou Y, et al. Common variants in mismatch repair genes associated with increased

risk of sperm DNA damage and male infertility. BMC Med 2012;10:49.
17. Okuda H, Tsujimura A, Irie S, et al. A single nucleotide polymorphism within the novel sex-linked testis-specific retrotransposed PGAM4 gene influences human male fertility. PLoS One 2012;7(5):e35195.
18. Tuttelmann F, Simoni M, Kliesch S, et al. Copy number variants in patients with severe oligozoospermia and Sertoli-cell-only syndrome. PLoS One 2011;6(4):e19426.
19. Lopes AM, Aston KI, Thompson EE, et al. Human spermatogenic failure purges deleterious mutation load from the autosomes and both sex chromosomes, including the gene DMRT1. PLoS Genet 2013;9(3):e1003349.
20. Choi Y, Jeon S, Choi M, et al. Mutations in SOHLH1 gene associate with nonobstructive azoospermia. Hum Mutat 2010;31(7):788–93.
21. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod 1999;14(1):131–5.
22. Nicopoullos JD. The results of 154 ICSI cycles using surgically retrieved sperm from azoospermic men. Hum Reprod 2004;19(3):579–85.
23. Hansen M, Kurinczuk JJ, Milne E, et al. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. Hum Reprod Update 2013;19(4):330–53.
24. Kong A, Frigge ML, Masson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. Nature 2012;488(7412):471–5.
25. Wang J, Fan HC, Behr B, et al. Genome-wide single-cell analysis of recombination activity and de novo mutation rates in human sperm. Cell 2012;150(2):402–12.
26. Kovac JR, Pastuszak AW, Lamb DJ. The use of genomics, proteomics, and metabolomics in identifying biomarkers of male infertility. Fertil Steril 2013;99(4):998–1007.
27. De Jonge C. Semen analysis: looking for an upgrade in class. Fertil Steril 2012;97(2):260–6.
28. Lipshultz LI, Howards SS, Niederberger CS. Infertility in the male. Cambridge (United Kingdom): Cambridge University Press; 2010.
29. Alvarez C, Castilla JA, Martinez L, et al. Biological variation of seminal parameters in healthy subjects. Hum Reprod 2003;18(10):2082–8.
30. Hotaling JM, Walsh TJ. Male infertility: a risk factor for testicular cancer. Nat Rev Urol 2009;6(10):550–6.
31. Pietiläinen KH, Kaprio J, Vaaralahti K, et al. Circulating anti-Mullerian hormone levels in adult men are under a strong genetic influence. J Clin Endocrinol Metab 2012;97(1):E161–4.
32. Kuijper EA, Lambalk CB, Boomsma DI, et al. Heritability of reproductive hormones in adult male twins. Hum Reprod 2007;22(8):2153–9.
33. Myers RM, Stamatoyannopoulos J, Snyder M, et al. A user's guide to the encyclopedia of DNA elements (ENCODE). PLoS Biol 2011;9(4):e1001046.
34. Daniel L, Hartl AG. Principles of population genetics. 4th edition. Sunderland (MA): Sinauer Associates; 2007.
35. Kosova G, Abney M, Ober C. Colloquium papers: heritability of reproductive fitness traits in a human population. Proc Natl Acad Sci U S A 2010;107(Suppl 1):1772–8.
36. Galanter J, Choudhry S, Eng C, et al. ORMDL3 gene is associated with asthma in three ethnically diverse populations. Am J Respir Crit Care Med 2008;177(11):1194–200.
37. Bell CJ, Dinwiddie DL, Miller NA, et al. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 2011;3(65):65ra64.
38. Bojesen A, Gravholt CH. Klinefelter syndrome in clinical practice. Nat Clin Pract Urol 2007;4(4):192–204.
39. Vogt PH, Falcao CL, Hanstein R, et al. The AZF proteins. Int J Androl 2008;31(4):383–94.
40. Vogt PH. AZF deletions and Y chromosomal haplogroups: history and update based on sequence. Hum Reprod Update 2005;11(4):319–36.
41. Gibson G. Hints of hidden heritability in GWAS. Nat Genet 2010;42(7):558–60.
42. Clarke AJ, Cooper DN. GWAS: heritability missing in action? Eur J Hum Genet 2010;18(8):859–61.
43. Schultz N, Hamra FK, Garbers DL. A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. Proc Natl Acad Sci U S A 2003;100(21):12201–6.
44. Yu J, Chen Z, Ni Y, et al. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. Hum Reprod 2011;27(1):25–35.
45. Attardo T, Vicari E, Mollica F, et al. Genetic, andrological and clinical characteristics of patients with congenital bilateral absence of the vas deferens. Int J Androl 2001;24(2):73–9.
46. Samli H, Samli MM, Yilmaz E, et al. Clinical, andrological and genetic characteristics of patients with congenital bilateral absence of vas deferens (CBAVD). Arch Androl 2006;52(6):471–7.
47. Southern KW. Cystic fibrosis and formes frustes of CFTR-related disease. Respiration 2007;74(3):241–51.
48. Zielenski J. Genotype and phenotype in cystic fibrosis. Respiration 2000;67(2):117–33.
49. Wilschanski M, Dupuis A, Ellis L, et al. Mutations in the cystic fibrosis transmembrane regulator gene and in vivo transepithelial potentials. Am J Respir Crit Care Med 2006;174(7):787–94.
50. Claustres M, Guittard C, Bozon D, et al. Spectrum of CFTR mutations in cystic fibrosis and in

congenital absence of the vas deferens in France. Hum Mutat 2000;16(2):143–56.
51. Uzun S, Gokce S, Wagner K. Cystic fibrosis transmembrane conductance regulator gene mutations in infertile males with congenital bilateral absence of the vas deferens. Tohoku J Exp Med 2005; 207(4):279–85.
52. Claustres M. Molecular pathology of the CFTR locus in male infertility. Reprod Biomed Online 2005;10(1):14–41.
53. Bareil C, Guittard C, Altieri JP, et al. Comprehensive and rapid genotyping of mutations and haplotypes in congenital bilateral absence of the vas deferens and other cystic fibrosis transmembrane conductance regulator-related disorders. J Mol Diagn 2007;9(5):582–8.
54. Shapiro E, Goldfarb DA, Ritchey ML. The congenital and acquired solitary kidney. Rev Urol 2003; 5(1):2–8.
55. McCallum T, Milunsky J, Munarriz R, et al. Unilateral renal agenesis associated with congenital bilateral absence of the vas deferens: phenotypic findings and genetic considerations. Hum Reprod 2001;16(2):282–8.
56. Male Infertility Best Practice Policy Committee of the American Urological Association, Practice Committee of the American Society for Reproductive Medicine. Report on evaluation of the azoospermic male. Fertil Steril 2006;86(5 Suppl 1): S210–5.
57. Kamal A, Fahmy I, Mansour R, et al. Does the outcome of ICSI in cases of obstructive azoospermia depend on the origin of the retrieved spermatozoa or the cause of obstruction? A comparative analysis. Fertil Steril 2010;94(6): 2135–40.
58. Liu J, Lissens W, Silber SJ, et al. Birth after preimplantation diagnosis of the cystic fibrosis delta F508 mutation by polymerase chain reaction in human embryos resulting from intracytoplasmic sperm injection with epididymal sperm. JAMA 1994;272(23):1858–60.
59. Chandley AC. Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility. Hum Reprod 1998;13(Suppl 1): 45–50.
60. Yoshida A, Miura K, Nagao K, et al. Sexual function and clinical features of patients with Klinefelter's syndrome with the chief complaint of male infertility. Int J Androl 1997;20(2):80–5.
61. Yoshida A, Miura K, Shirai M. Cytogenetic survey of 1,007 infertile males. Urol Int 1997;58(3):166–76.
62. Lanfranco F, Kamischke A, Zitzmann M, et al. Klinefelter's syndrome. Lancet 2004;364(9430):273–83.
63. Oates RD. Clinical and diagnostic features of patients with suspected Klinefelter syndrome. J Androl 2003;24(1):49–50.
64. Wikstrom AM, Dunkel L, Wickman S, et al. Are adolescent boys with Klinefelter syndrome androgen deficient? A longitudinal study of Finnish 47, XXY boys. Pediatr Res 2006;59(6):854–9.
65. Groth KA, Skakkebaek A, Host C, et al. Clinical review: Klinefelter syndrome–a clinical update. J Clin Endocrinol Metab 2013;98(1):20–30.
66. Swerdlow AJ, Schoemaker MJ, Higgins CD, et al. Cancer incidence and mortality in men with Klinefelter syndrome: a cohort study. J Natl Cancer Inst 2005;97(16):1204–10.
67. Swerdlow AJ, Hermon C, Jacobs PA, et al. Mortality and cancer incidence in persons with numerical sex chromosome abnormalities: a cohort study. Ann Hum Genet 2001;65(Pt 2):177–88.
68. Aguirre D, Nieto K, Lazos M, et al. Extragonadal germ cell tumors are often associated with Klinefelter syndrome. Hum Pathol 2006;37(4):477–80.
69. Arnedo N. Sperm aneuploidy in fathers of Klinefelter's syndrome offspring assessed by multicolour fluorescent in situ hybridization using probes for chromosomes 6, 13, 18, 21, 22, X and Y. Hum Reprod 2005;21(2):524–8.
70. Foresta C, Galeazzi C, Bettella A, et al. Analysis of meiosis in intratesticular germ cells from subjects affected by classic Klinefelter's syndrome. J Clin Endocrinol Metab 1999;84(10):3807–10.
71. Bakircioglu ME, Erden HF, Kaplancan T, et al. Aging may adversely affect testicular sperm recovery in patients with Klinefelter syndrome. Urology 2006; 68(5):1082–6.
72. Aksglaede L, Wikstrom AM, Rajpert-De Meyts E, et al. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. Hum Reprod Update 2006;12(1):39–48.
73. Vicdan K, Akarsu C, Vicdan A, et al. Birth of a healthy boy using fresh testicular sperm in a patient with Klinefelter syndrome combined with Kartagener syndrome. Fertil Steril 2011;96(3):577–9.
74. Okada H, Goda K, Muto S, et al. Four pregnancies in nonmosaic Klinefelter's syndrome using cryopreserved-thawed testicular spermatozoa. Fertil Steril 2005;84(5):1508.e13–6.
75. Hinney B, Guttenbach M, Schmid M, et al. Pregnancy after intracytoplasmic sperm injection with sperm from a man with a 47, XXY Klinefelter's karyotype. Fertil Steril 1997;68(4):718–20.
76. Bourne H, Stern K, Clarke G, et al. Delivery of normal twins following the intracytoplasmic injection of spermatozoa from a patient with 47, XXY Klinefelter's syndrome. Hum Reprod 1997;12(11): 2447–50.
77. Komori S, Horiuchi I, Hamada Y, et al. Birth of healthy neonates after intracytoplasmic injection of ejaculated or testicular spermatozoa from men with nonmosaic Klinefelter's syndrome: a report of 2 cases. J Reprod Med 2004;49(2):126–30.

78. Maiburg M, Repping S, Giltay J. The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. Fertil Steril 2012;98(2):253–60.
79. Ramasamy R, Reifsnyder JE, Husseini J, et al. Localization of sperm during microdissection testicular sperm extraction in men with nonobstructive azoospermia. J Urol 2013;189(2):643–6.
80. Sigman M. Klinefelter syndrome: how, what, and why? Fertil Steril 2012;98(2):251–2.
81. Vorona E, Zitzmann M, Gromoll J, et al. Clinical, endocrinological, and epigenetic features of the 46, XX male syndrome, compared with 47, XXY Klinefelter patients. J Clin Endocrinol Metab 2007; 92(9):3458–65.
82. Schiebel K, Winkelmann M, Mertz A, et al. Abnormal XY interchange between a novel isolated protein kinase gene, PRKY, and its homologue, PRKX, accounts for one third of all (Y+)XX males and (Y-)XY females. Hum Mol Genet 1997;6(11): 1985–9.
83. Rajender S, Rajani V, Gupta NJ, et al. SRY-negative 46, XX male with normal genitals, complete masculinization and infertility. Mol Hum Reprod 2006; 12(5):341–6.
84. Patrat C, Bienvenu T, Janny L, et al. Clinical data and parenthood of 63 infertile and Y-microdeleted men. Fertil Steril 2010;93(3):822–32.
85. Yatsenko AN, Yatsenko SA, Weedin JW, et al. Comprehensive 5-year study of cytogenetic aberrations in 668 infertile men. J Urol 2010;183(4):1636–42.
86. Lange J, Skaletsky H, van Daalen SK, et al. Isodicentric Y chromosomes and sex disorders as byproducts of homologous recombination that maintains palindromes. Cell 2009;138(5):855–69.
87. Van Assche E, Bonduelle M, Tournaye H, et al. Cytogenetics of infertile men. Hum Reprod 1996; 11(Suppl 4):1–24 [discussion: 25–6].
88. Mau-Holzmann UA. Somatic chromosomal abnormalities in infertile men and women. Cytogenet Genome Res 2005;111(3–4):317–36.
89. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003;423(6942):825–37.
90. Navarro-Costa P. Sex, rebellion and decadence: the scandalous evolutionary history the human Y chromosome. Biochim Biophys Acta 2012; 1822(12):1851–63.
91. Simoni M, Tuttelmann F, Gromoll J, et al. Clinical consequences of microdeletions of the Y chromosome: the extended Munster experience. Reprod Biomed Online 2008;16(2):289–303.
92. Repping S, Skaletsky H, Lange J, et al. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet 2002;71(4):906–22.
93. Repping S, Skaletsky H, Brown L, et al. Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet 2003;35(3):247–51.
94. Viswambharan N, Suganthi R, Simon AM, et al. Male infertility: polymerase chain reaction-based deletion mapping of genes on the human chromosome. Singapore Med J 2007;48(12):1140–2.
95. Carvalho CM, Zuccherato LW, Bastos-Rodrigues L, et al. No association found between gr/gr deletions and infertility in Brazilian males. Mol Hum Reprod 2006;12(4):269–73.
96. Reijo R, Alagappan RK, Patrizio P, et al. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996;347(9011):1290–3.
97. Reijo R, Lee TY, Salo P, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995;10(4):383–93.
98. Ferlin A, Arredi B, Speltra E, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. J Clin Endocrinol Metab 2007;92(3): 762–70.
99. Mau Kai C, Juul A, McElreavey K, et al. Sons conceived by assisted reproduction techniques inherit deletions in the azoospermia factor (AZF) region of the Y chromosome and the DAZ gene copy number. Hum Reprod 2008;23(7):1669–78.
100. Nuti F, Krausz C. Gene polymorphisms/mutations relevant to abnormal spermatogenesis. Reprod Biomed Online 2008;16(4):504–13.
101. Vogt PH. Azoospermia factor (AZF) in Yq11: towards a molecular understanding of its function for human male fertility and spermatogenesis. Reprod Biomed Online 2005;10(1):81–93.
102. Lavery R, Glennon M, Houghton J, et al. Investigation of DAZ and RBMY1 gene expression in human testis by quantitative real-time PCR. Arch Androl 2007;53(2):71–3.
103. Ferlin A, Moro E, Rossi A, et al. The human Y chromosome's azoospermia factor b (AZFb) region: sequence, structure, and deletion analysis in infertile men. J Med Genet 2003;40(1):18–24.
104. Ferlin A, Raicu F, Gatta V, et al. Male infertility: role of genetic background. Reprod Biomed Online 2007;14(6):734–45.
105. Visser L, Westerveld GH, Korver CM, et al. Y chromosome gr/gr deletions are a risk factor for low semen quality. Hum Reprod 2009;24(10): 2667–73.
106. Yang Y, Ma M, Li L, et al. Differential effect of specific gr/gr deletion subtypes on spermatogenesis in the Chinese Han population. Int J Androl 2010; 33(5):745–54.

107. Kee K, Angeles VT, Flores M, et al. Human DAZL, DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation. Nature 2009;462(7270):222–5.
108. Teng YN, Lin YM, Sun HF, et al. Association DAZL haplotypes with spermatogenic failure in infertile men. Fertil Steril 2006;86(1):129–35.
109. Vodicka R, Vrtel R, Dusek L, et al. TSPY gene copy number as a potential new risk factor for male infertility. Reprod Biomed Online 2007; 14(5):579–87.
110. Su MT, Chen CH, Kuo PH, et al. Polymorphisms of estrogen-related genes jointly confer susceptibility to human spermatogenic defect. Fertil Steril 2010; 93(1):141–9.
111. Lee IW, Kuo PH, Su MT, et al. Quantitative trait analysis suggests polymorphisms of estrogen-related genes regulate human sperm concentrations and motility. Hum Reprod 2011;26(6):1585–96.
112. Castro-Nallar E, Bacallao K, Parada-Bustamante A, et al. Androgen receptor gene CAG and GGN repeat polymorphisms in Chilean men with primary severe spermatogenic failure. J Androl 2010;31(6): 552–9.
113. Grigorova M, Punab M, Zilaitiene B, et al. Genetically determined dosage of follicle-stimulating hormone (FSH) affects male reproductive parameters. J Clin Endocrinol Metab 2011;96(9): E1534–41.
114. Carrell DT. Epigenetics the male gamete. Fertil Steril 2012;97(2):267–74.
115. Hammoud SS, Nix DA, Zhang H, et al. Distinctive chromatin in human sperm packages genes for embryo development. Nature 2009;460(7254): 473–8.
116. Hammoud S, Liu L, Carrell DT. Protamine ratio and the level of histone retention in sperm selected from a density gradient preparation. Andrologia 2009; 41(2):88–94.
117. Hammoud SS, Purwar J, Pflueger C, et al. Alterations in sperm DNA methylation patterns at imprinted loci in two classes of infertility. Fertil Steril 2010;94(5):1728–33.
118. OBrienFlynn KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril 2010;93(1):1–12.
119. Dunham I, Kundaje A, Aldred SF, et al. An integrated encyclopedia of DNA elements in the human genome. Nature 2012;489(7414):57–74.
120. Sanyal A, Lajoie BR, Jain G, et al. The long-range interaction landscape of gene promoters. Nature 2012;489(7414):109–13.
121. Thurman RE, Rynes E, Humbert R, et al. The accessible chromatin landscape of the human genome. Nature 2012;489(7414):75–82.
122. Hotaling JM, Lopushnyan NA, Davenport M, et al. Raw and test-thaw semen parameters after cryopreservation among men with newly diagnosed cancer. Fertil Steril 2013;99(2):464–9.
123. Fisch H, Hyun G, Golden R, et al. The influence paternal age on down syndrome. J Urol 2003; 169(6):2275–8.
124. Cooper TG, Noonan E, von Eckardstein S, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16(3):231–45.
125. Franken DR, Oehninger S. Semen analysis and sperm function testing. Asian J Androl 2012; 14(1):6–13.
126. Hotaling JM, Davenport MT, Eisenberg ML, et al. Men who seek infertility care may not represent the general U.S. population: data from the National Survey of Family Growth. Urology 2012;79(1):123–7.
127. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th edition. Geneva (Switzerland): WHO; 2010. Available at: http://whqlibdoc.who.int/publications/2010/9789241547789_eng.pdf.
128. Hammiche F, Laven JS, Twigt JM, et al. Body mass index and central adiposity are associated with sperm quality in men of subfertile couples. Hum Reprod 2012;27(8):2365–72.
129. Schoor RA, Elhanbly S, Niederberger CS, et al. The role of testicular biopsy in the modern management of male infertility. J Urol 2002;167(1):197–200.
130. Ashok S, Sigman M. Bioavailable testosterone should be used for the determination of androgen levels in infertile men. J Urol 2007;177(4):1443–6.
131. Sussman EM, Chudnovsky A, Niederberger CS. Hormonal evaluation of the infertile male: has it evolved? Urol Clin North Am 2008;35(2):147–55.
132. Anawalt BD, Hotaling JM, Walsh TJ, et al. Performance of total testosterone measurement to predict free testosterone for the biochemical evaluation of male hypogonadism. J Urol 2012; 187(4):1369–73.
133. Kosova G, Scott NM, Niederberger C, et al. Genome-wide association study identifies candidate genes for male fertility traits in humans. Am J Hum Genet 2012;90(6):950–61.
134. Hotaling JM, Kosova G, Ohlander S, et al. Genome-wide association study identifies candidate genes for abnormal sperm morphology in two cohorts of men. American Urological Association 2013 Annual Meeting. San Diego, CA, May 4–8, 2013.
135. Brandes M, Hamilton CJ, van der Steen JO, et al. Severity of oligo-asteno-teratozoospermia no longer determines overall success rate in male subfertility. Int J Androl 2011;34(6 Pt 1):614–23.