The genetic causes of male factor infertility: A review

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Objective: To illustrate the necessity for an enhanced understanding of the genetic basis of male factor infertility, to present a comprehensive synopsis of these genetic elements, and to review techniques being utilized to produce new insights in fertility research.

Background: Male factor infertility is a complex disorder that affects a large sector of the population; however, many of its etiologies are unknown. By elucidating the underlying genetic basis of infertile phenotypes, it may be possible to discover the causes of infertility and determine effective treatments for patients.

Method(s): The PubMed database was consulted for the most relevant papers published in the last 3 years pertaining to male factor infertility using the keywords "genetics" and "male infertility."

Result(s): Advances have been made in the characterization of the roles of specific genes, but further research is necessary before these results can be used as guidelines for diagnosing and treating male factor infertility. The accurate transmission of epigenetic information also has considerable influence on fertility in males and on the fertility of their offspring.

Conclusion(s): Analysis of the genetic factors that impact male factor infertility will provide valuable insights into the creation of targeted treatments for patients and the determination of the causes of idiopathic infertility. Novel technologies that analyze the influence of genetics from a global perspective may lead to further developments in the understanding of the etiology of male factor infertility through the identification of specific infertile phenotype signatures. (Fertil Steril® 2010;93:1–12. ©2010 by American Society for Reproductive Medicine.)

Key Words: Male infertility, genetics, Y chromosome, epigenetics, AZF region

Infertility affects approximately 15% of couples (1), and genetic abnormalities are thought to account for 15%–30% of male factor infertility (2). Genetics contributes to infertility by influencing a variety of physiological processes including hormonal homeostasis, spermatogenesis, and sperm quality. Therefore, an understanding of the genetic basis of reproductive failure is essential to appropriately manage an infertile couple. Considered as one of the most perplexing disorders in the reproductive field, male factor infertility is prevalent, and its incidence is rising while its etiology remains elusive. This paper will discuss the genetic causes of male factor infertility that are considered most relevant today.

METHODS

An exhaustive literature review was performed in PubMed using the keywords "male infertility" and "genetics." The results were filtered by limiting the search to English manuscripts published within the last 3 years that discussed studies of human subjects. This initial search produced 646 associated articles. Subsequent searches were performed

using the keywords "Y chromosome," "epigenetics," "genomics," "proteomics," and "metabolomics" to further supplement the information obtained. After careful review of the abstracts, 40 articles were selected for inclusion in the manuscript. This group of articles consisted of one meta-analysis, 19 original articles, and 20 review articles.

THE IMPORTANCE OF ELUCIDATING THE GENETIC BASIS

The advent of assisted reproductive techniques (ART) has emphasized the necessity for clinicians to recognize the role that genetics plays in male factor infertility cases because new technologies, such as intracytoplasmic sperm injection (ICSI), allow men with suboptimal sperm quality to overcome natural selection mechanisms and produce a viable zygote (2). Because ART, like in vitro fertilization (IVF) and ICSI, involves relatively new procedures, the frequency of the inheritance of mutations through these procedures and their impact on future generations are not yet fully understood (3). Some andrologists have voiced concern about concealing reproductive defects through ART that might have negative consequences at the epigenetic level (4, 5), but currently there is no definite evidence of imprinting disorders associated with the procedures (3). Although the majority of children conceived through ART seem normal, a slight increase was noted in the prevalence of aneuploidy in the sex chromosomes of ICSI children (from 0.2% to 0.6%). In addition, there were increased autosomal chromosome abnormalities (from 0.07% to 0.4%) (6). However, these data are difficult to

interpret because patients who use ICSI or other ART have a higher incidence of abnormalities due to their infertile status. The development of techniques to test for genetic abnormalities or unfavorable polymorphisms before performing ART is critical.

Consequently, it is necessary to determine the underlying genetic basis of male factor infertility to develop appropriate screens for abnormal phenotypes and to discover more effective solutions for infertile couples' problems (7). Abnormalities in the genetic message passed on through ART can have serious implications for the developing zygote.

GENETIC CAUSES
Chromosomal Abnormalities

Chromosomal abnormalities account for approximately 5% of infertility in males, and the prevalence increases to 15% in the population of azoospermic males (2). Abnormalities of the Y chromosome, such as microdeletions, are the major cause of azoospermia (the complete absence of sperm in the ejaculate) and severe cases of oligozoospermia (less than 20 million sperm/mL) (8, 9). Therefore, chromosomal errors are a pertinent area of research to determine the role of genetics in male factor infertility. Table 1 provides a quick reference of the abnormalities discussed in the following paragraphs.

Aneuploidy, or incorrect chromosome number, is the most common error resulting from chromosomal abnormalities in infertile men (10). Men with nonobstructive azoospermia have a particularly high incidence of aneuploidy (11), especially in their sex chromosomes (12). Although aneuploid sperm have an altered amount of genetic material, occasionally they can successfully fertilize the oocyte and pass on an incorrect chromosome number to their offspring (7).

Klinefelter syndrome, the most common chromosomal abnormality caused by aneuploidy, has a prevalence of 5% in men with severe oligozoospermia and 10% in azoospermic men (13). The syndrome usually causes the arrest of spermatogenesis at the primary spermatocyte stage, but occasionally later stages of sperm development are observed (3). There are two forms of Klinefelter syndrome: nonmosaic, 47, XXY; and mosaic, 47, XXY/ 46, XY. Although previously believed to be sterile, it has been estimated that 25% of nonmosaic Klinefelter syndrome patients have sperm in their ejaculate (2). Men with the mosaic form of the disease may have residual spermatogenesis in their seminiferous tubules; the Foresta et al. 2005 study found that 74% of the men were azoospermic (13). Klinefelter syndrome patients may try to achieve pregnancy using ICSI, but they risk producing offspring with chromosomal abnormalities (14, 15). This fear was substantiated by several studies that observed that Klinefelter syndrome patients have large numbers of aneuploid gametes (3). However, some studies have produced successful results with nonmosaic men and ICSI (16, 17). It is advised that preimplantation genetic diagnosis (PGD) be performed before ART to ensure that the offspring is not aneuploid (3).

Chromosomal translocations are an additional source of aneuploidy (18). Translocations can cause the loss of genetic material at the break points of genes, which can corrupt the genetic message (7). Autosomal translocations were found to be 4–10 times more likely in infertile males in comparison with normal males (19, 20). Robertsonian translocations, which occur when two acrocentric chromosomes fuse, are the most frequent structural chromosomal abnormalities in humans, and they affect fertility in one out of 1000 men (2, 21). Although the prevalence of Robertsonian translocations is only 0.8% in infertile males, this figure is 9 times higher than in the general population (22). The translocations can result in a variety of sperm production phenotypes from normal spermatogenesis to an inability to produce spermatogonia (3). Robertsonian translocations are more common in oligozoospermic and azoospermic men, with rates of 1.6% and

0.09%, respectively (23, 24). Carriers of Robertsonian translocations may exhibit a normal phenotype but could be infertile because of a lack of gamete production (2). Because of the risk of passing on the translocation to offspring, fluorescent in situ hybridization, with additional probes added for common translocations, is recommended to determine the chromosomal composition of the sperm (3).

Y Chromosome

The Y chromosome is an obvious area of interest in the study of male factor infertility because it contains many of the genes that are critical for spermatogenesis and the development of male gonads. The variation present on the Y chromosome and the occurrence of deletions of large segments of the chromosome involving multiple genes make it difficult to determine the exact cause of certain infertile phenotypes (25). Furthermore, the same phenotype may be produced by several different deletions or mutations. This fact complicates efforts to distinctively correlate mutations with infertile phenotypes.

Y chromosome microdeletions are a frequent cause of infertility in males. A microdeletion is defined as a chromosomal deletion that spans several genes but is not large enough to be detected using conventional cytogenetic methods (26). Studies have revealed that microdeletions are more prevalent in men who are azoospermic and severely oligozoospermic (27). The prevalence of microdeletions in azoospermic men was found to range from 10%–15% (28, 29). In oligozoospermic men, the prevalence of microdeletions was 5%–10% (28). It is essential to consider these deletions when discussing ART because microdeletions are always passed on to the male offspring (30, 31) and fertilization and pregnancy rates are not affected by microdeletions on the AZFc region when using ICSI (31).

Microdeletions most frequently occur on the long arm of the Y chromosome, Yq, and deletions in this region are specifically related to failure of spermatogenesis (32, 33). A particular area of interest on Yq is the azoospermia factor region (AZF region), which contains genes involved in the growth and development of sperm. The AZF region contains three subregions: AZFa, AZFb, and AZFc (34). The most common aberrations that occur in the AZF region are multiple gene deletions in the AZFb and AZFc areas (9), which can produce a wide range of infertile phenotypes. Microdeletions in the AZF region are most often found in azoospermic and oligozoospermic men with normal karyotypes (34). Researchers are attempting to characterize deletions in the AZF region so that they can be used to determine treatment for infertile males (34). Figure 1 displays the different AZF regions of the Y chromosome and the locations of genes discussed in the following paragraphs.

AZFa The two main genes located in the AZFa region are *USP9Y* and *DBY* (also called *DDX3Y*) (2). Deletions in the AZFa region that remove both of these genes cause Sertoli cell–only syndrome, a condition characterized by the presence of complete Sertoli cells in the testes but a lack of spermatozoa in the ejaculate (34, 35). *DBY*, the major gene located in the AZFa region, has a probable role in infertility because it is localized in the testis and is involved in the development of premeiotic germ cells (34). The Lardone et al. study of the transcriptional activity of several AZF region genes found that men with Sertoli cell–only syndrome had reduced levels of *DBY* transcripts but that the other genes examined were transcribed normally. This finding suggests that *DBY* may play an important role in spermatogenesis, but further studies must be performed to replicate this result (36). The *USP9Y* gene is also involved in spermatogenesis (37). Shortening or deletion of the *USP9Y* gene causes azoospermia (67), oligozoospermia (38), or oligoasthenozoospermia (39). However, it seems that this gene may only be

Prevalence and phenotypes of common chromosomal abnormalities associated with male infertility.

Genetic abnormality	Phenotype	Prevalence, %
Chromosomal abnormalities	Azoospermia to normozoospermia	5 (total infertile population); 15 (azoospermic)
Klinefelter syndrome	Azoospermia to severe oligozoospermia	5 (severe oligozoospermia); 10 (azoospermic)
Robertsonian translocation	Azoospermia to normozoospermia	0.8 (total infertile population); 1.6 (oligozoospermic); 0.09 (azoospermic)
Y chromosome microdeletions	Azoospermia to oligozoospermia	10–15 (azoospermic); 5–10 (oligozoospermic)
AZFa deletion	Azoospermia, Sertoli cell–only syndrome	0.5–1.0 (2)
AZFb deletion	Azoospermia, spermatogenic arrest	0.5–1.0 (2)
AZFc deletion	Severe oligozoospermia to nonobstructive azoospermia	6–12
Partial AZF-c deletions	From azoospermia to normozoospermia	3–5 (2)

*Note:* Prevalence listed refers to listed phenotype unless noted otherwise.

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involved in the efficiency of spermatogenesis because it can be passed on to offspring. Furthermore, other animals, such as bonobos and chimpanzees, do not have active forms of the gene (37). These findings suggest that the *DBY* gene has a more critical role in spermatogenesis than the *USP9Y* gene. Further research studies must be performed to determine the exact roles of each of these genes in fertility to develop more targeted Y chromosome screening practices for infertile males (39).

AZFb Deletions of the AZFb region cause arrest of spermatogenesis at the primary spermatocyte stage (34), indicating that the region is essential for fertility (35). The main gene in the AZFb region is *RBMY*, and there are six copies of the gene located on the Y chromosome (33). *RBMY1* codes for an RNA binding protein (40), which is a testis-specific splicing factor expressed in the nuclei of spermatogonia, spermatocytes, and round spermatids (34). In the study by

FIGURE 1

Image of Y chromosome displaying AZF regions and associated genes. Enlarged portion of AZFc region highlights discussed microdeletions. (A) Normal AZFc region; (B) gr/gr deletion; (C) b1/b3 deletion; (D) g1/g3 deletion; (E) gr/gr duplication.
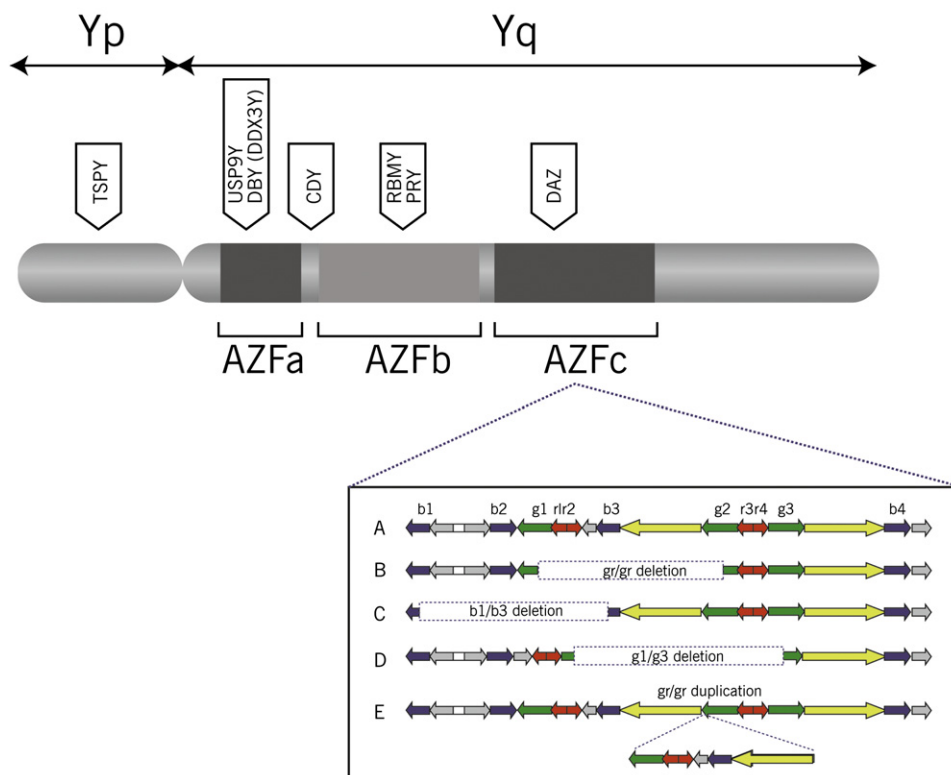
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TABLE 2

Ethnic variation of gr/gr mutations.

Variation	Effect	Ethnicity	Study
Deletion	Failure of spermatogenesis	Dutch	Repping et al. 2003 (169)
Deletion	Failure of spermatogenesis	Spanish	de Llanos et al. 2005 (170)
Deletion	Failure of spermatogenesis	Italian	Giachini et al. 2005 (171); Ferlin et al. 2005 (172)
Deletion	Failure of spermatogenesis	Australian	Lynch et al. 2005 (173)
Deletion	No correlation	French	Machev et al. 2004 (174)
Deletion	No correlation	German	Hucklenbroich et al. 2005 (175)
Deletion	No correlation	Brazilian	Carvalho et al. 2006 (176)
Deletion	No correlation	Japanese	de Carvalho et al. 2006 (177)
Deletion	No correlation	Chinese	Zhang et al. 2006 (178)
Deletion	No correlation	Sri Lankan	Fernando et al. 2006 (179)
Duplication	Risk for impaired spermatogenesis	Han Chinese	Lin et al. 2007 (52)

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Lavery et al., *RBMY1* expression was reduced in azoospermic men (41). 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 (34). In cases in which all the genes in the AZFb region except *RBMY* and *PRY* are deleted, patients present with hypospermatogenesis (42). However, if both the *RBMY* and *PRY* genes are removed, spermatogenesis is arrested completely (43), indicating that *RBMY* and *PRY* are the major genes involved in fertility in the AZFb region.

AZFc Deletions in the AZFc region produce a wide range of phenotypes, many of which are associated with low sperm concentration due to reduced spermatogenesis (34). AZFc deletions cause approximately 12% of nonobstructive azoospermia and 6% of severe oligozoospermia (44). In many cases, men can still achieve fertilization with the assistance of ART (34). Studies demonstrate that only the AZFa and AZFb regions are needed to initiate spermatogenesis but that without the AZFc region, spermatogenesis will not be completely normal (3). Complete deletions of the AZFc region may occur in two different ways: either as a result of a previous deletion within the AZFc or spontaneously from a normal AZFc region. A study by Zhang et al. found that there were more complete deletions of the AZFc region in groups with existing partial deletions in that area of the Y chromosome (45). This result was replicated in a study of Italian men with a high frequency of partial deletions in the AZFc region (46). A deletion of the AZFc region may also predispose men to Y chromosome loss, leading to sexual reversal. Several studies have found this deletion to be a premutation for 45,X0 (47, 48) and for the mosaic phenotype 45,X/46,XY (49).

The AZFc region is prone to many smaller subdeletions that are thought to be caused by intrachromosomal recombinations (2). These partial deletions produce a wide array of phenotypes, ranging from normospermic to azoospermic, due to many factors, including the interaction of the environment and the genetic background. Genetic studies of ethnic groups produce diverse results because of the variations in their genomes that have evolved over generations to cope with environmental pressures specific to their region (50). Therefore, studies of the partial deletions of the AZFc region have produced conflicting results that relate to the genetic makeup of the haplogroups studied. The complex interaction of genes and environment makes it difficult to replicate the results of studies and definitively associate subdeletions with infertile phenotypes (2). The three most frequent subdeletions on the AZFc region of the Y chromosome are gr/gr, b1/b3, and g1/g3 (b2/b3) (51). The gr/gr subdeletion, which removes half of the content in the AZFc region, illustrates the complicating effects of ethnic background on gene function. Several studies have identified the deletion as a risk factor for the loss of spermatogenesis, while others failed to find a correlation (45). Furthermore, a study of the Han Chinese population discovered that duplication of the gr/gr region was detrimental to fertility, contributing to further uncertainty about the role that this region plays in determining a man's fertility status (52). This finding has not yet been replicated in other studies. Table 2 summarizes these studies according to geographic region to illustrate the complex effects of environment and genetics on fertility.

The AZFc region also contains genes involved in spermatogenesis. The *DAZ* gene has four copies on the Y chromosome (53). *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 (25). They regulate translation, code for germ cell–specific RNA binding proteins (54), and are involved in the control of meiosis and maintenance of the primordial germ cell population (25). Deletions of the *DAZ* genes can cause a spectrum of phenotypes ranging from oligozoospermia to azoospermia (55). Additionally, *DAZ* gene expression was reduced in azoospermic patients (41), and partial deletions of *DAZ* genes seem to be related to oligozoospermia (56).

It is critical that azoospermic and severely oligozoospermic men be tested for microdeletions both for accurate diagnosis and genetic counseling before performing ART (57, 58). However, the lack of association between testicular phenotype and genotype in affected men forces clinicians to employ inefficient and costly methods, such as polymerase chain reaction (PCR), to determine diagnosis. The Y chromosome contains 300 sequence tagged sites (STS), which correspond to the AZF regions and could be exploited for easier characterization of microdeletions (28). Mitra et al. demonstrated the utility of this strategy by developing a targeted multiplex PCR using STS specific to the Indian population. This type of procedure could be used as an initial screen for Y chromosome microdeletions before employing more expensive and technically challenging testing methods (59). However, to be effective, specific STS would need to be defined for different ethnic populations.

Other Genes on the Y Chromosome Another gene involved in spermatogenesis and located on Yq is *CDY*, the chromodomain protein Y-linked gene. It is expressed exclusively in the testis and is involved in facilitating the replacement of histones in spermatogenesis. It also

grants the proteins that regulate transcription easier access to the postmeiotic sperm DNA through the acetylation of histones (34). The *CDY* gene seems to have diverged functionally from its autosomal homologue (*CDYL*, located on chromosome 6) during evolution and then subsequently migrated to the Y chromosome. This fact makes it an interesting gene to study and demonstrates that there may be a tendency for genes with spermatogenic function to consolidate on the Y chromosome (34).

The *TSPY* gene is located on the short arm of the Y chromosome, Yp, and it also has copies on the long arm of the chromosome (36). The gene is expressed in the testis, and its protein has been identified in spermatogonia (60). The *TSPY* gene may regulate the timing of spermatogenesis by signaling spermatogonia to enter meiosis (35). A study of copy number variation of the *TSPY* gene found that more copies were found in infertile patients (61). This finding warrants further investigation of *TSPY* to characterize its role in infertility.

Table 3 is a visual representation of the information presented above to serve as an easy reference for the reader.

Autosomal Gene Mutations and Polymorphisms

Many autosomal genes are also being investigated for possible roles in male factor infertility. The *CFTR* gene, located on chromosome 7 (62), is mutated in 60%–90% of patients with congenital bilateral absence of the vas deferens (CBAVD) (2, 3). CBAVD is a form of obstructive azoospermia in which there is a disconnection between the epididymis and the ejaculatory duct that causes a functional block to natural fertilization. Men with CBAVD usually either have two mild mutations in the *CFTR* gene or the combination of a severe mutation and a mild mutation. The most common severe mutation, F508del, is found in 60%–70% of patients with CBAVD (3). ICSI is a useful method of treatment for men with the *CFTR* mutation as long as the female does not also carry the *CFTR* mutation (2). Partners who both carry the mutation should be advised to have PGD to avoid passing the abnormality to their offspring (3).

The sex hormone–binding globulin (*SHBG*) gene, located on chromosome 17, has also been studied for a possible role in spermatogenesis. The gene is involved in both delivering sex hormones to target tissues and controlling the concentration of androgens in the testis (63). Androgens play important roles in sexual differentiation and the process of spermatogenesis; if androgen levels are disrupted, fertility could decrease. A study that examined the effects of the SHBG(TAAAA)n polymorphism on male factor fertility concluded that shorter *SHBG* alleles were associated with increased levels of spermatogenesis and higher sperm concentration. Shorter *SHBG* alleles were related to elevated levels of circulating SHBG, resulting in higher levels of free androgens to stimulate the spermatogenetic process (63). This study by Lazaros et al. employed a small sample size; consequently, a larger population of subjects should be examined to confirm the relevance of their findings to the field of infertility.

Other autosomal genes that have been investigated for a possible involvement in fertility are the estrogen receptor genes *ESR1* and *ESR2* (35). Studies have found an association between abnormal spermatogenesis and estrogen insufficiency, prompting the investigation of the *ESR* genes (35). *ESR1*, found on chromosome 6, has several different polymorphisms that have been studied for their role in male factor infertility, especially in relation to severe oligozoospermia, and the results have been varied (64). This variation is most likely due to the interactions of the genes and the environment because the variations are mostly between different ethnic groups. The promoter region of *ESR1* also has a variable number of tandem repeats, (TA)n (65). The (TA)n polymorphism is related to sperm output: a higher number of repeats on both alleles is correlated with lower levels of spermatogenesis. Sperm production might be negatively affected by the elevated numbers of repeats because it is thought that they result in lower levels of estrogens and increased estrogen activity (35). The effect of this polymorphism was found to be similar in both fertile and infertile Italian men, although the result was not statistically significant in the fertile population (65). The correlation with sperm output presents the (TA)n polymorphism as a candidate for further studies. The *ESR1* also contains the AGATA haplotype, which is caused by five single nucleotide polymorphisms (SNPs) located within the gene (35). A recent study in the Japanese population identified this haplotype as a risk factor for cryptorchidism (66), but this finding was not replicated in Italian and Spanish populations (67). In the *ESR2* gene located on chromosome 14, the *RsaI* polymorphism has been examined, but studies have produced conflicting evidence (64, 68). Both Nuti and Krausz and Tuttelmann et al. suggested that the *ESR* genes be examined further to replicate the results of previous uncoordinated studies to clarify the significance of these mutations on male factor fertility (35, 68).

The FSH receptor (*FSHR*) gene is also being studied for involvement in spermatogenesis. The gene is located on chromosome 2, and it codes for the receptor for FSH, an essential hormone for normally functioning gonads (64, 68). One study found that partial deletions of the *FSHR* gene had slight effects on spermatogenesis (69). Additionally, a *FSHR* SNP has been discovered that may affect the activity of the gene (2). Preliminary studies by Simoni et al. (70) and Ahda et al. (71) have both found differences in the *FSHR* polymorphisms between fertile and infertile men. These initial results

TABLE 3

Genes on Y chromosome with suspected involvement in male factor infertility.

Gene	Location	Reasons for investigation
USP9Y	AZFa	Involved in efficiency of spermatogenesis; deletion or shortening may cause azoospermia, oligozoospermia, or oligoasthenozoospermia
DBY	AZFa	Involved in premeiotic germ cell development
RBMY	AZFb	RNA binding protein/testis-specific splicing factor; reduced expression in azoospermic men
PRY	AZFb	Regulation of apoptosis
DAZ	AZFc	Regulation of translation, meiosis, and germ cell population; codes for RNA binding proteins; reduced expression in azoospermic men; partial deletions related to oligozoospermia
CDY	Yq	Involved in histone replacement
TSPY	Yp	Regulates timing of spermatogenesis; greater copy number in infertile patients

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suggest that the *FSHR* gene should be studied further to characterize its involvement in fertility.

The autosomal homologue of the *DAZ* gene, *DAZL*, is another gene that is still being studied owing to inconclusive results. The gene is located on chromosome 3 and codes for the RNA binding proteins involved in the regulation of protein expression and meiosis (64, 68). Two different SNPs, one each at exon 2 and exon 3, have been discovered. The SNP at exon 3 is only associated with infertility in populations of Chinese men (72), but this finding has not been replicated (35). A study by Tung et al. identified four new mutations in the *DAZL* gene, but further studies are necessary to determine their effects (73). Tung et al. also identified haplotypes in the *DAZL* gene related to sperm count (74), and Teng et al. discovered haplotypes associated with failure of the spermatogenic process (75). The findings of these uncoordinated studies suggest that further investigation of the *DAZL* gene must be performed to elucidate the role that the gene plays in infertility.

The *MTHFR* (methylenetetrahydrofolate reductase) gene, located on the short arm of chromosome 1 (64), codes for an enzyme involved in folate metabolism, a critical factor in DNA methylation and the spermatogenetic process (35). The polymorphism 677C → T causes the substitution of an alanine for a valine, which decreases the activity of the enzyme (76). The reduced activity of *MTHFR* can lead to the dysregulation of folic acid metabolism, causing errors in the methylation of genomic DNA and subsequent implications in spermatogenesis (35). The polymorphism is related to infertility in African, South East Asian, and Indian men (77, 78), but these results were not replicated in the European populations that were studied (2). Further studies must be performed to confirm the role of this gene in fertility, although it seems likely that the *MTHFR* gene is involved due to its influence on spermatogenesis (68).

Cryptorchidism is another infertile phenotype that seems to be influenced by genetic factors. Mutations in the *INSL3* gene (insulin-like 3 on chromosome 19) and its receptor *LGR8* (relaxin/insulin-like family peptide receptor 2 on chromosome 13) (2, 64) have been linked to cryptorchidism (79). These mutations occur in approximately 5% of men with cryptorchidism (80). Additionally, the first phase of normal testicular descent is controlled by *INSL3* (81, 82). The *INSL3* gene may also have a role in testicular dysgenesis syndrome (TDS) (83), which consists of a variety of disorders like cryptorchidism, hypospadias, testicular cancer, and infertility. It is thought that TDS results from the combination of genetic, environmental, and lifestyle factors (79).

X-Linked Genes

Many X-linked genes are expressed in the testis (84) and are thought to be involved in gametogenesis (35). The androgen receptor (*AR*) gene is located on the long arm of the X chromosome (35). It plays a role in meiosis and the conversion of spermatocytes to round spermatids during spermatogenesis (85). A recent study of infertile men determined that approximately 2% had mutations in their *AR* gene, while the control population had none (86). Mutations of the *AR* gene can also lead to androgen insensitivity syndrome (2). Androgen insensitivity results from mutations that impede the ability of androgens to bind to their receptor and from decreased transactivation potential (35). Another possible result of mutations in the *AR* gene is Kennedy syndrome, a neurodegenerative disorder characterized by abnormalities in spermatogenesis (87).

The *AR* gene also has two polymorphisms that have been studied for their role in male factor infertility. The CAG and GGC polymorphisms, both located on exon 1, code for polyglutamine and polyglycine stretches, respectively (2). Examinations of the role of the GGC polymorphism have produced limited findings. Although it seems to have an inverse relationship with the transactivation activity of the receptor (88), no significant difference in the lengths of the GGC polymorphisms has been determined between infertile men and the general population (88, 89). The CAG polymorphism has been studied more intensively than the GGC polymorphism. Longer lengths of the CAG polymorphism are associated with decreased transcriptional activity of the *AR* gene in infertile men, suggesting that longer polyglutamine tracts are related to male factor infertility (2). Some researchers also observed that shorter CAG polymorphisms were related to higher quality sperm and increased levels of spermatogenesis (63). Conversely, Lazaros et al. discovered a correlation between short CAG repeat length and sperm motility, but there was no observed effect on sperm concentration (63). This study also identified a synergistic effect of the *SHBG* gene and the *AR* gene that influences sperm motility (63). These polymorphisms may be affected by ethnic influences as well because studies performed in Europe failed to find a correlation between the CAG polymorphism and infertility (90), while studies in men from Asia (91), Singapore (88), and Australia (92) found a relationship. These results warrant further exploration of the role of the CAG polymorphism.

The *USP26* gene is also located on the long arm of the X chromosome and is expressed throughout the testes in the preliminary stages of spermatogenesis (93). It is thought to be involved in histone removal during spermatogenesis and the breakdown and reformation of proteins (35). Studies have found a relationship between the gene and infertility, for example, the discovery of variations of the gene in azoospermic men (93, 94). Also, three SNPs have been identified that were thought to be related to spermatogenic failure. However, this finding was challenged by the discovery of the group in a man with normal spermatogenesis (95). The effects of the SNPs also seem to be influenced by ethnicity; they are relatively common in some groups of African and Asian men (96).

The *TAF7L* gene has also been studied as a possible contributor to infertility in men. It is expressed in the testis and is related to the autosomal *TAF7* gene, which is a transcription factor (35). Transcription factor regulators play integral roles in spermatogenesis because they control the spatial and temporal aspects essential for accurate execution of the process (97, 98). A study by Falender et al. found that *TAF* genes were involved in spermatogenesis in the mouse (99), prompting the examination of the role of *TAF7L* in human spermatogenesis by Akinloye et al. (100). The study discovered that an SNP at exon 13 may be a risk factor for an infertile phenotype if combined with additional polymorphisms or mutations (100). This finding warrants additional research to elucidate the role of this gene in the genetic basis of male factor infertility (100, 101).

Kallmann syndrome (KS) is another genetic condition that can cause infertility in males and has both X-linked and autosomal genetic components. KS is defined as idiopathic hypogonadotropic hypogonadism (IHH) combined with anosmia or hyposmia. The disorder is caused by a defect in the migration of the GnRH neurons (102). IHH is characterized by low levels of sex steroids in combination with low to normal levels of FSH and LH (103). Patients can either be afflicted with complete or incomplete IHH, which leads to a range of stages of sexual development (104). The absence or low levels of sex steroids inhibit or stunt sexual development and spermatogenesis in males. In addition to reduced sexual development, KS patients have cognitive impairments, ocular abnormalities, midfacial clefting, and renal agenesis. Two genetic deletions found to be specifically related to KS patients are *KAL1* (KS 1 sequence) and *FGFR1* (fibroblast growth factor receptor 1) (64, 102). *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 (102). *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 KS. This variation in phenotype indicates the reduced penetrance of the deletion within the population (102). The discovery of the causes of KS is clinically relevant because it can help clinicians and researchers understand the proper development of the neuroendocrine axis and regulation of pubarche.

From the above discussion, it is evident that much progress has been made in the identification of candidate genes with suspected involvement in male factor infertility. Unfortunately, clinically relevant applications of these discoveries have not been discovered at the same rate (35). Additionally, inconclusive findings from individual studies and from the complex effects of ethnicity complicate the analysis of the role of individual genes. Both Nuti and Krausz and Tuttelmann et al. advocated the need for large multicenter studies with appropriate controls, strong statistical power, and large cohorts to test the findings of specific case-control studies (35, 68). Studies identifying genetic mutations thus far should serve as a launch point for these larger investigations of male factor infertility. In future studies, researchers should strive to limit studies according to narrow phenotypes and characteristic associated symptoms. For instance, they should avoid conducting studies including both azoospermic and oligozoospermic men, even if they are studying the same genetic mutation, to try to eliminate confounding factors (68). Additionally, global approaches to the study of infertility (discussed in the Novel Technologies section) are currently being developed, which provide promising leads for more effective methods to diagnose and treat infertility.

EPIGENETIC ERRORS

Spermatogenesis is a complex series of events vulnerable to the accumulation of errors that can severely affect the spermatogenic process (3). Additionally, sperm must be correctly packaged and programmed to successfully pass on genetic and epigenetic information to the developing embryo. Epigenetics refers to alterations of the genetic code that do not affect the basic DNA sequence, such as the addition of different molecules to the DNA, which changes the regulation of transcription and, consequently, gene expression (105).

One important contribution that the sperm makes to the embryo is a functional centrosome. The centrosome is involved in the process of fertilization, the separation of chromosomes, and cell division (106). Rawe et al. observed that a patient with abnormal centrosome morphology and sperm aster formation had difficulty fertilizing oocytes. Furthermore, if a pregnancy was achieved, the fetus was aborted (107). Sperm harvested from the testicles before maturation may not have a fully functional centrosome, which could lead to problems with the segregation of chromosomes and result in a mosaic or aneuploid embryo (108, 109). Abnormal centrosomes may also be related to the failure of the gametes to unite properly, another error that may cause cleavage arrest (107).

As mentioned earlier, chromatin packaging is an essential step in sperm development, and it is believed that the compacted structure of the chromatin transmits vital instructions to the embryo to guide it through development (110). During chromatin repackaging, 85% of the histones are replaced by protamines (111–113). In an intermediate step in the replacement process, transitional proteins are inserted into the chromatin structure (114). Studies in mice revealed that disruption of the genes that code for the transitional proteins TP1 and TP2 can produce infertile phenotypes (115, 116). Additionally, the functions of the two different human protamines, P1 and P2, have been elucidated. If the mRNA of P1 is translated too early, spermatogenesis is arrested at the spermatid stage and the nucleus condenses

prematurely (117). P2 has been found to be directly related to sperm DNA damage and abnormally packaged chromatin structure (118). Furthermore, if either of the genes that code for P1 or P2 is mutated, haploinsufficiency, abnormalities in the structure of the chromatin, DNA damage, and infertility can occur (35). An unequal ratio of P1 and P2 has been observed in infertile men (119). Men with unequal protamine ratios have decreased semen quality, decreased fertilization ability, and DNA damage (112, 120). Abnormal protamine ratios may also be associated with problems in epigenetic reprogramming and gene imprinting (111) and have been correlated with IVF success and embryo quality (121). An SNP, G197 T, was found in the gene that codes for P1. This SNP may be a factor that predisposes men to DNA fragmentation and teratozoospermia or to a high prevalence of morphologically abnormal sperm in the ejaculate (122).

Histones are another important contributor to the transmission of epigenetic information. Histone markers signify DNA imprinting control regions during the formation of spermatozoa (123, 124). The transcriptional control of gene expression is regulated by the addition of acetyl, methyl, ubiquitin, and phosphate groups to histones (125). Abnormally modified histones are probable candidates for impeding normal embryogenesis, and their role in the fertility is currently under investigation (114).

Imprinting, the methylation of DNA, determines which genes from the parental and maternal genomes are expressed in the embryo (10) and is critical for normal development (126). The imprinted regions of DNA are reset every reproductive cycle (126), which allows novel parental imprints to be established on the germ cells (127). Imprinting is achieved by the differential marking of DNA regions with histone modifications, methylation, or possibly both, to allow only one copy of a gene to remain active (128).

Kobayashi et al. performed a study examining the fidelity of imprint resetting in infertile males. In fertile men with a normal ejaculate, paternal differentially methylated regions (DMRs) of the DNA should be methylated and the maternal DMRs should be unmethylated. The study found that approximately 14% of infertile patients had abnormalities in the DMRs of the paternal imprint and 21% of the infertile patients had abnormalities in the maternal imprint. Most patients with abnormalities in both imprint regions were oligozoospermic. Additionally, men with abnormally imprinted DMRs had low success rates with ART. It was also discovered that oligozoospermic men may have a higher risk of transmitting imprinting errors to their children (126). ART could have negative consequences on the imprinting of sperm because it may use sperm that are not yet fully mature and, consequently, their epigenetic code is not established. If the sperm are too immature or abnormal, it is more likely that the offspring could be born with an imprinting disorder (10). Other defects associated with fertilization by immature sperm are centrosome abnormalities (129), abnormalities in the sperm's nuclear protein, or an inability to activate the oocyte (130). The control of methylation may also be a point at which dysregulation could occur. Studies in knockout mice for DNA methyltransferases produced males that were oligozoospermic; however, this has not been replicated in humans (126).

Thus far, studies examining the global methylation patterns of sperm from infertile men have not found significant differences in comparison with normal men, but additional research is necessary to definitively confirm the role of imprinting and epigenetic information in infertility (121). The correlation between the incidence of imprinting disorders and ART in men with abnormal sperm is a controversial topic. A study by Hartmann et al. found that spermatogonia from infertile men did not have increased imprinting errors in comparison with that of normal men (131). In contrast, other

researchers have asserted that ART, such as ICSI, causes imprinting disorders like Angelman syndrome and Beckwith-Wiedemann syndrome. Angelman syndrome is a rare neurological disorder characterized by cognitive defects, seizures, uncontrolled limb and body movements, spontaneous laughter, and difficulties with speech development (132, 133). Two independent groups reported an increased incidence of Angelman syndrome in offspring from ICSI procedures (4, 134). Beckwith-Wiedemann syndrome, also an uncommon disorder, is characterized by large fetal and organ size, hypoglycemia, midline abdominal defects, facial moles, and enlarged tongues (132). Children with Beckwith-Wiedemann syndrome are also at risk for developing tumors (135). A study by DeBaun et al. reported that the incidence of Beckwith-Wiedemann syndrome was almost 5% in children conceived by ART in comparison with an incidence of less than 1% in the general population (136). Other studies also reported increased rates of Beckwith-Wiedemann syndrome in ICSI children (137). However, in consideration of the studies that have not found increased rates of imprinting errors in infertile men, these results are intriguing. Further research into the abnormalities caused by imprinting errors and their patterns of inheritance is needed in this contentious field of research.

The sperm delivers mRNA transcripts to the oocyte upon fertilization, which are needed for the correct development of a functional embryo (114) and which transmit epigenetic information (110). The fact that these mRNA transcripts are necessary for normal development is emphasized by their presence in zygotes (138). Furthermore, there is also evidence that the phenotypic characteristics of the embryo might be influenced by the mRNA contributed by the sperm (138). Studies of the expression profiles of infertile males' mRNA are currently being performed (as discussed in the Novel Technologies section). Micro-RNA (miRNA) is also present in human sperm and in the early embryonic stages (139). It is possible that miRNA is involved in the control of gene expression in the embryo (140, 141); however, miRNA has been found in high concentrations in the oocyte, so its significance as a sperm contribution still remains controversial (142).

Telomeres have also been examined as potential candidates for the production of infertile phenotypes. Telomeres protect the genetic information encoded on the chromosome, localize the chromosomes in the nucleus, and play a role in DNA replication (10). Abnormal shortening of the telomeres has been associated with male factor infertility (143). Hemann et al. performed a study of telomere length in knockout mice for telomerase, the enzyme that maintains the length of telomeres (144). The results imply that there is a mechanism that degrades spermatocytes with reduced telomere length to prevent their maturation (144). However, the process is not flawless; Liu et al. discovered spermatocytes that reached meiosis I with shortened telomeres, indicating that they passed the checkpoint without being degraded (145). Additionally, studies of telomere length in different infertile phenotypes, including nonobstructive and obstructive azoospermic patients and oligozoospermic patients, did not report significant differences in telomerase activity (146). Thus, the influence of telomere length on male factor fertility must be further elucidated (10).

Mitochondrial DNA (mtDNA) inheritance may also impact male factor infertility. Abnormal mitochondria cause problems in sperm motility because of aberrations in the mitochondrial sheath (147). There is concern of passing mtDNA abnormalities to offspring using ART such as ICSI because the entire sperm is injected into the oocyte and the mtDNA of the sperm is conserved. Furthermore, oligozoospermic males have been found to have higher rates of mtDNA mutations (148), and various studies have found a correlation between abnormalities in mtDNA and dysfunctional sperm (149,

150). Other studies have produced conflicting data that complicate the role of mtDNA abnormalities in male factor infertility. A study by Marchington et al. reported that paternal mtDNA could not be identified in ICSI children (151). This finding confirms the presumption that paternal mtDNA is degraded after fertilization (152).

Clinically, both direct methods, such as single-cell gel electrophoresis, and indirect methods, such as sperm chromatin integrity assays, have been used to determine the quality of sperm DNA (153). However, existing data from studies of DNA integrity and pregnancy outcomes in ART and natural conception have not confirmed a correlation. Consequently, there is currently no functional role for DNA integrity testing as a predictor of fertility (153).

NOVEL TECHNOLOGIES

Adopting a global approach to the examination of novel genes may allow for a more complete understanding of the interaction between genetics and fertility and could uncover genes with unknown roles in infertility. This approach may also circumvent one of the main problems that geneticists face when relating a genotype to a specific infertility phenotype: the diverse genetic backgrounds of different ethnic groups (154). Incorporating techniques such as genomics, proteomics, and metabolomics into infertility research could assist in creating a complete portrait of the genes involved in infertility and would allow for improvements of ART for the development of more targeted solutions.

Microarrays are valuable tools for the identification of gene expression profiles of infertile phenotypes (155). Examining the simultaneous expression of genes allows geneticists to determine molecular signatures related to infertile phenotypes (156). Microarray technology is also useful in the examination of spermatogenesis. An analysis of gene expression over time could be performed to determine the genes that are involved in each stage of the process. Genomic analysis can also be used to determine differentially transcribed genes (157). An enhanced understanding of transcription regulation could help geneticists discover how different expression patterns impact a patient's fertility (155). Additionally, microarrays can be used to study the effect of hormones or growth factors on gene expression profiles. In one experiment, T propionate and FSH were administered to mouse testes and the differential expression of genes was measured using microarray analysis (156). Some advantages of using microarrays are that it is a noninvasive test (158) and it is very effective in studying germ cells because they express 4% of the genome (159). Disadvantages of genomics are that gene expression can vary between two different samples (154) and infertile patients might have pockets of gene expression that are difficult to detect using microarrays (160).

Ellis et al. performed a study of several different infertile phenotypes that revealed two distinct patterns of gene expression, one related to spermatogenesis and one related to inflammatory activity (155). They identified functional groups within the gene expression patterns related to spermatid development and motility, DNA synthesis and repair, metabolic functions, and cholesterol and lipid metabolism (155). The study also discovered a correlation between infertile phenotypes and mRNA expression (155). Another study of gene expression was performed in teratozoospermic men by Platts et al. which identified characteristic mRNA signatures for teratozoospermic and normospermic men (155, 158). Since the results of microarray studies of gene expression produce variable results, it is necessary to determine global gene expression patterns of RNA samples from the testis before this type of analysis would be clinically relevant. Grouping the expressed genes into functional categories allows for the characterization of a gene expression

signature for normal human spermatogenesis that can be used as a baseline marker for diagnosis (2).

Proteomics allows for the determination of protein expression profiles of fertile and infertile men (161). Proteins are identified with two-dimensional electrophoresis and mass spectrometry techniques, and the results are used to create maps of the proteome (161). Spermatozoa are ideal for the study of protein expression because they do not have active transcription or translation (162). Further research in these fields can continue after the sperm proteome is fully defined and the components of seminal plasma are identified (162). Advances have been made in both of these areas (163–165), and many new proteins have been identified as a result. The identification of protein biomarkers for male factor infertility will allow for unbiased comparison between fertile and infertile males and will clarify the pathophysiology of the disease (162).

Martinez-Heredia et al. studied asthenozoospermic patients using proteomic techniques. Most of the causes of asthenozoospermia, or abnormally low sperm motility levels, are unknown, even though it is a common infertile phenotype (161). The study found 17 differentially expressed proteins between the control group and the asthenozoospermic patients. The results were clustered, signifying that results from proteomic studies could possibly be used to characterize or diagnose infertile patients. Subgroupings of functional groups were also determined from the results and were divided into energy production, structure/movement, and intercell signaling. These subgroups could be used to target the underlying causes of asthenozoospermia (161).

An advantageous characteristic of genomic and proteomic technology is that the results can be confirmed through replication using other techniques such as Western blots, flow cytometry, and PCR. Without reproducible results, a test is meaningless, especially with multistep procedures like these that may accumulate errors (156). Another important feature of these tests is that the results provide a definitive characterization of infertile phenotypes (158). The use of technologies like genomics and proteomics is a step toward creating personalized medical diagnoses by determining individual causes of infertility (158).

Metabolomics is another emerging area of research in the evaluation of the role of genetic factors in male factor infertility. Metabolomics involves measuring the expression of metabolites, small biomarkers that indicate the functionality of a cell, and characterizing them for certain diseases or physiological states (166, 167). The identification of the human metabolome will reveal the functional phenotype of the system being studied, whether it is a single cell or an entire organism. Mass spectroscopy, nuclear magnetic resonance spectroscopy, and other chromatography methods can be used to create profiles of metabolites. Pathway or cluster analysis is used to determine subsets of metabolites that can be used to quantitatively characterize patients for diagnosis (168). By identifying differences in the expression of metabolites in infertile phenotypes, new methods of diagnosis and treatment of male factor infertility can be developed that are inexpensive and noninvasive (166). Thus far, metabolomics has been used to identify biomarkers for oxidative stress (OS), which signal semen quality (166). A study performed by Deepinder et al. determined that expression patterns of metabolomic markers for OS in semen were correlated to specific infertile phenotypes with a high level of specificity and sensitivity (166). This encouraging finding may assist in unraveling the underlying mysteries that still surround many cases of idiopathic male factor infertility. Other future clinical applications of metabolomics are gamete selection (assessing the best sperm to use for ART) and functional genomic testing (screening for aneuploidy and other genetic conditions) (166). Next, efficient clinical methods must be developed to compare standardized metabolomic signatures with patients' personal metabolomic profiles for the creation of individualized fertility care (168). These novel technologies hold promise for advances in the ways in which information about genetic profiles can aid infertility patients.

CONCLUSION

Although much work still must be completed to fully determine the involvement of genes in the production of infertile phenotypes, current research findings suggest that accurate transmission of genetic and epigenetic information is essential for fertility. Through the efforts to link genetic mutations to specific infertile phenotypes and the use of global investigative approaches such as metabolomics, researchers may be able to determine the interactions of genetics, environment, and ethnic background on fertility. Using this knowledge, clinicians will be better able to treat infertile patients and make knowledgeable decisions about the use of ART.

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