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Understanding the genes involved in spermatogenesis: a progress report

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Objective: To review the current literature on genes known to affect fertility in the human and mouse.

Design: A literature review was performed and key articles were chosen for focus in the areas of genes with effects only on spermatogenesis and oogenesis, with an emphasis on Y-chromosome–encoded gene families and spermatogenesis. In addition, studies describing genes deleted in transgenic mice were incorporated.

Result(s): Several gene families on the Y chromosome are implicated in spermatogenic failure, but the link between the genetic lesion and the resulting defect is unclear. Many mouse genes involved in repair and DNA damage monitoring have specific effects on gametogenesis in and around meiosis.

Conclusion(s): Many genes are involved only in gametogenesis, and some of these are beginning to be understood in terms of their functions. An even larger number of genes is required for gametogenesis, and other functions and mouse models give insights important for human disease. (Fertil Steril® 1998;69:989–95. ©1998 by American Society for Reproductive Medicine.)

Key Words: Genes, RNA, meiosis, splicing, translation, repair

Between 2% and 12% of couples worldwide are affected by reduced fertility (1). Men who are infertile account for about half of these cases, and a large proportion of these men are infertile either because of insufficient sperm (oligozoospermia) or lack of sperm (azoospermia). The cause of these defects in sperm production is unclear, but recent work points to both potential environmental and genetic causes. The explosive growth in the use of intracytoplasmic sperm injection (ICSI) and related techniques focuses our attention particularly on genetic components of spermatogenic impairment because these could be transmitted at higher frequency by these procedures than by unassisted fertilization.

Spermatogenesis is a unique process of continuing differentiation because, unlike processes such as hematopoiesis, the DNA content of the product is half that of the progenitor cells. In the initial stages, spermatogonia undergo mitotic divisions, giving rise to primary and secondary spermatocytes, the cell type in which the first and second meiotic divisions occur. The haploid products of meiosis are the round spermatids, which elongate during the process called spermiogenesis and compact their chromatin into the sperm head and produce the other sperm components. Despite the interest in the processes involved in this pathway, we know little about them.

This review focuses on recent developments in the genetics of male infertility, with an emphasis on the clinical relevance of current knowledge and on the likely directions that research into the basic biology will take.

THE Y CHROMOSOME IS NOT ESSENTIAL FOR LIFE

Genes central to the process of spermatogenesis cannot be identified easily by family studies or mapped by linkage approaches because of the limitations imposed on family size by the nature of the defect. The Y chromosome presents a unique situation in this context. Not only is it thought to be a favored location for genes involved in male physiology for evolutionary reasons based on the concept of genetic hitchhiking, but it is also dispensable for normal development of the female. In effect, this means that individuals with deletions of the Y chromosome can be viable.

Genes located on the Y chromosome are

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studied easily because they exist in a haploid state. Furthermore, it is unlikely that Y-chromosome microdeletions propagated by new technology such as ICSI will have harmful effects other than on fertility. As a result of the absence of genetic recombination, mapping of genes on the Y chromosome outside the pseudoautosomal regions has relied on the analysis of cytogenetic deletions and structural abnormalities.

AZOOSPERMIA FACTOR: NOT A SINGLE GENE

An important early observation was that some azoospermic men had deletions of the distal long arm of the Y chromosome, suggesting that an underlying genetic defect may be responsible in some cases (2). This led to the postulation of a locus encoding the azoospermia factor (or *AZF*) in this region of the Y chromosome, a gene essential for normal sperm production. Recent results aimed at mapping this locus in azoospermic men with much smaller Y-chromosome deletions (microdeletions) have complicated this picture substantially and suggest that rather than a single locus, three nonoverlapping regions of the Y-chromosome long arm are important for male fertility. These regions are now called *AZFa-c* (3) (Fig. 1). Candidate genes have been isolated from *AZFb* and *AZFc,* and, surprisingly, both encode distinct RNA-binding proteins.

ISOLATION OF GENES IMPORTANT FOR SPERMATOGENESIS

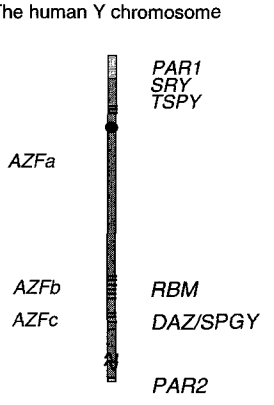
RBM was the first of these candidate genes to be identified (4). Rather than being encoded by a single gene, there is a family of 20–50 RBM genes and pseudogenes spread over both arms of the Y chromosome, including a cluster within the *AZFb* region (5). Notably, RBM encodes a protein (we will refer to the protein product of genes with a p; thus, the protein encoded by RBM is RBMp, the gene RBM) with a single RNA recognition motif, a sequence of amino acids shown to bind RNA. Its expression is restricted to the male germline in man (6) and mouse (7). RBM is closely related to the autosomal hnRNPG (8, 9), and both are members of the hnRNP family of proteins, which are associated with nuclear polyadenylated RNA and are involved in pre-mRNA packaging, transport to the cytoplasm, and splicing (10). However, an important difference is that hnRNPG is ubiquitously expressed, suggesting that its function is required in all cell types.

GENES PRESENT IN MANY COPIES ARE HARD TO ANALYZE

The multicopy nature of the RBM gene family in human (and mouse) has complicated attempts to prove an essential role for the protein in spermatogenesis (5, 7, 11). An important question has been whether all the RBM family members

FIGURE 1

The cartography of the human Y chromosome. PAR1 and PAR2 are the pseudoautosomal regions, SRY are the testis-determining genes, and TSPY is the major location of a multigene family encoding a protein of unknown function.



with disparate Y-chromosomal locations are functionally redundant. Recently, studies using antibodies to RBM protein for immunocytochemical analysis of the testes of infertile men with Y-chromosome deletions localized (6) the active copies to the *AZFb* region, as defined by Vogt et al. (3). However, it remains possible that the deletion of genes other than RBM that lie within this region may be partly or wholly responsible for causing the observed spermatogenic arrest.

DAZ: ANOTHER MULTICOPY GENE FAMILY

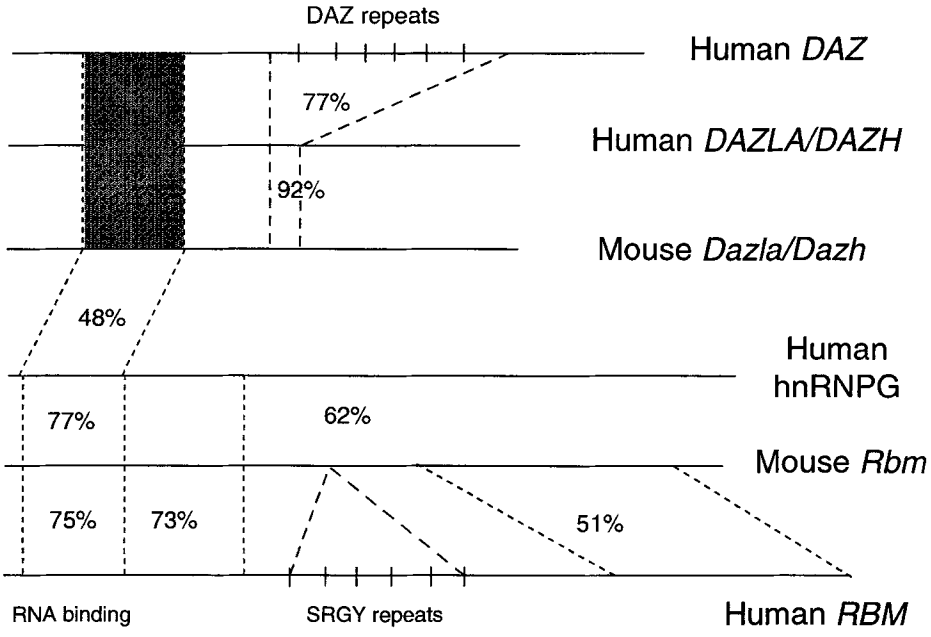
More recently, two other AZF candidate genes have been isolated from the Y chromosome. Although distinct in sequence from RBM, both DAZ (12) and SPGY (13) encode proteins with a similar modular structure, with a single RNA recognition motif and an internally repeated sequence (Fig. 2). Originally, DAZ was thought to be single copy, whereas SPGY was multicopy. It is now clear that both are members of the same gene family, which maps in the distal part of the Y-chromosome euchromatin in a region corresponding to Vogt's *AZFc* (13).

The best estimate of the number of genes in the family is 6–10. This region of the Y chromosome is deleted in 4–13% of patients presenting at infertility clinics with either azoospermia or severe oligozoospermia (14–17). This frequency of de novo deletion is the highest yet reported for any region of the human genome and presumably reflects structural features of the DNA or chromatin in this region of the Y chromosome.

The existence of oligozoospermic individuals with DAZ/SPGY deletions implies that these genes are not absolutely required for the completion of spermatogenesis, a conclusion

FIGURE 2

Amino acid homologies between different parts of a number of RNA-binding proteins. Those for the DAZ and daz-related genes were from ref. 22.



strengthened by the observation that at least one man deleted for DAZ/SPGY fathered a son who inherited the microdeletion (3). It is puzzling that this son was azoospermic, which suggests that other factors may affect the penetrance of this deletion. This theory would be consistent with the diverse testicular phenotypes observed in men with *AZFc* deletions. The recent advances in assisted conception using ICSI also have implications for the subsequent fertility of any male progeny who will inherit their father's deleted Y chromosome (16, 18), as this may perpetuate genetic causes of infertility.

Y-CHROMOSOME GENES AND EVOLUTION

Conservation of a protein through evolution and the degree of relatedness of the same genes between species are commonly thought to be indicators of the functional pressure on a gene. RBM genes are present on the Y chromosomes of all mammalian species tested, including marsupials (19). Conservation of RBM on the Y chromosome suggests selection, as otherwise genes would tend to degenerate rapidly because of the lack of recombination on this chromosome.

DAZ/SPGY is derived from an autosomal gene, DAZLA/*Dazh*, first described in mice on chromosome 17 close to or within the distal end of a region called the *t* complex (20, 21). This is intriguing because, if within the *t* complex, it could be a candidate for one of many fertility factors mapped to this region of the mouse genome. In an important recent advance, several laboratories (22–25) have shown that there is also a human autosomal homologue of DAZ/SPGY, called DAZ*LA* or DAZ*H* or SPGY*LA*. This nomenclature nightmare is being resolved; all SPGY genes will become DAZ and all autosomal homologues will be designated DAZL.

In the human genome, DAZL is on chromosome 3. Despite their autosomal location, the DAZL genes of both mouse and man are expressed only in the germline. These differences in the distribution of RBM and DAZ/SPGY genes may reflect the same mode of evolution, the acquisition of fertility genes by the Y chromosome, but with very different timing of the events. Gene family RBM is derived from an hnRNPG-like ancestor and is copied to the Y chromosome before the radiation of mammals (19); in the case of the DAZL gene family, this process occurred much more recently in the old-world primate lineage. In the absence of recombination, a functional gene cannot be restored by recombination between two nonfunctional copies, resulting in the one-way accumulation of mutations (26).

The multicopy nature of both gene families on the Y chromosome compared with their single-copy autosomal relatives could be explained as an escape mechanism from this process, which could maintain a functional gene. Alternatively, selection imposed by chromosome position may also act to increase the copy number of the DAZ/SPGY family, which is close to the heterochromatin of the long arm of the human Y chromosome, if these heterochromatic re-

TABLE 1

Autosomal genes with roles in gametogenesis.

Gene	Protein type	Localization	Phenotype of mutant
Dazl	RNA binding	Premeiotic cytoplasm	Male and female infertility
HR6B	Ubiquitin-conjugating enzyme	Spermatids	Male infertility
BMP 8B	Signaling (TGF-β-like)	Round spermatids	Failure of germ cell development
Dhh	Signaling	Sertoli cells	Failure of germ cell development
Hsp70-2	Heat-shock protein	Synaptonemal complex	Male infertility
Pms2	Mismatch repair		Male infertility
Mlh1	Mismatch repair	Synaptonemal complex	Male and female infertility, growth retardation
ATM	Check point/kinase	Synaptonemal complex	Male and female infertility
ATR	Check point/kinase	Synaptonemal complex	?

gions exert a silencing effect on nearby genes. Amplification may distance copies of the gene from the heterochromatin and so relieve silencing. The result could be an increased copy number of the genes. There may also simply be a requirement for a large amount of the message or multiple variants of the protein.

IMPLICATIONS OF EVOLUTION

Although deletion of DAZ has been detected in 5–10% of men with severe oligozoospermia or azoospermia, this figure varies widely among studies, possibly because of differences in populations or in criteria for inclusion in the studies. In contrast, point mutations have not been detected in either DAZ or DAZL. If there are no other genes involved in these deletions, this implies that the Y-linked DAZ/SPGY genes in humans have a function differentiated from the DAZL genes, as these genes would be present in men with Y-chromosome deletions. This functional separation must have arisen recently because mammals other than old-world primates can achieve spermatogenesis without Y-linked DAZ genes (23). Transcription of DAZL is gonad-specific, rather than testis-specific, suggesting that it could have a role in gametogenesis, which is shared between sperm and eggs.

The situation is similar for the functional RBM loci in that point mutations have not been detected. Initial studies suggested that these genes have a lower frequency of deletion than the DAZ genes, but more recent studies suggested that this may not necessarily be the case (27). Larger studies are needed to establish the relative frequencies with accuracy.

THE FUNCTIONS OF RBMp AND DAZp

What might the functions of these RNA-binding proteins be in spermatogenesis? RBMp is a nuclear protein (6), and it is tempting to speculate that, like hnRNPA1p, it could play a role in regulating splicing (28). Many testis-specific alternative splicing events have been described, and it is possible that RBMp-dependent splicing events could be essential for spermatogenesis. In the case of DAZL, its close *Drosophila* homologue *Boule* (29) is essential for progression through meiosis, suggesting that possible target RNAs could encode proteins required in meiosis. RNA-binding proteins are also critical for translational control in spermatogenesis, in which mRNAs are synthesized and then masked for translation at a later stage within the elongating spermatid, which is itself transcriptionally inactive (30). In this latter case, translational repression seems to be mediated in the cytoplasm, which would exclude the involvement of RBM, but not DAZ or DAZL (31).

A further possibility, suggested by the structural similarities of these RNA-binding proteins and the range of phenotypes observed in patients with apparently common deletions, is that the proteins may exhibit some functional overlap. A consequence of this might be that the effect of a deletion of, for example, DAZ/SPGY could be dependent on the alleles of DAZL or RBM present in that individual. Some of these questions can be addressed by genetics, and we have shown recently that in the mouse, the Dazlp is essential for gametogenesis in both sexes (31). The biochemistry and cell biology of these proteins in spermatogenesis require further study.

GENES WITH FUNCTIONS IN THE TESTIS AND ELSEWHERE

We have focused so far on Y-chromosome loci with effects only on spermatogenesis, but recent methods for manipulating the mouse genome in transgenic animals are starting to yield large numbers of other genes with involvement in spermatogenesis (Table 1). One approach to identifying and subsequently analyzing mouse genes with specific and essential roles in spermatogenesis has been to use recombination in cell culture to disrupt genes known to be specifically expressed or overexpressed in spermatogenesis and to generate "knockout" mice from these cells.

This approach has revealed several genes that are key players in normal spermatogenesis. For example, evidence for the important role of ubiquitinylation in spermatogenesis

has come from homozygous male mice disrupted for the autosomally encoded ubiquitin-conjugating enzyme mHR6B (32). These enzymes transfer activated ubiquitin to a ubiquitin-conjugating enzyme before transfer onto the target protein that is to be degraded. The resulting mice are infertile but are otherwise viable. The human HR6B and the closely related HR6A protein can ubiquitinylate histones, like the homologous RAD6 gene in *S. cerevisiae.*

Because postmeiotic condensation of chromosomes in round spermatids is defective in these mice, it has been proposed that mHR6A is involved in the replacement of histones with transition proteins before genome repackaging with protamines. Consistent with this, mHR6B is expressed in round spermatids, but also to a lesser extent in Sertoli cells and indeed in other tissues as well, suggesting an additional role for this protein outside of the testis (33). Why are the effects of the mHR6B disruption limited to the testis? One possibility is that expression of the similar mHR6A gene is enough to compensate for the lack of mHR6B expression in other tissues.

Mice with homozygous disruptions of two genes encoding signaling molecules, bone morphogenetic protein 8B (BMP 8B) and desert hedgehog (dhh), are infertile as a result of the absence or arrested development of germ cells (to some extent, the precise defect is dependent on genetic background) (34, 35). These mice are otherwise viable, showing that cell-cell interactions are critical for normal spermatogenic development. BMP 8B, a member of the transforming growth factor-β family, is expressed and secreted at low levels by spermatogonia at puberty, and then at much higher levels in round spermatids in the adult testis. The gene is thought to be involved in autocrine interactions between germ cells and/or short-range paracrine interactions between germ cells and Sertoli cells. In contrast, dhh is secreted by Sertoli cells from early embryogenesis to adulthood and is thought to mediate an interaction between Sertoli and Leydig cells.

PROTEINS WITH SYNAPTONEMAL COMPLEX INTERACTIONS

Genes of other types have been implicated as essential for spermatogenesis in mice. Targeted disruption of the heat shock protein *Hsp70-2,* which is specifically expressed in meiotic spermatocytes, results in a failure of meiosis and male infertility, possibly through an effect on synaptonemal complex formation (36). A second heat shock protein, *Hsc70tp*, is specifically expressed in round spermatids (37, 38).

One of the most intriguing sets of genes involved in meiosis are the mismatch repair genes Pms2 and Mlh1. These are hotly investigated because of the association between their biochemistry and malignancy, but when *Pms2* (39) and *Mlh1* (40, 41) are homozygously deleted, they have effects on chromosome pairing in meiosis. In the case of *Mlh1,* this results in male and female sterility, with the males producing no sperm and the females producing reduced numbers of oocytes. The gene product localizes to discrete foci on paired axes of the synaptonemal complexes at junctions between paired and unpaired regions. *Pms2* homozygotes are fertile as females but sterile as males.

Other proteins with functions in meiosis are ATM and ATR. The ATM gene was originally isolated as the gene mutated in ataxia-telangiectasia. It is involved in DNA metabolism and checkpoint control. It is closely related to the ATR gene, which is the human counterpart of the yeast S. pombe Rad3 gene. This gene is required for cell-cycle checkpoint pathways that respond to DNA damage and replication blocks in *S. pombe.*

Deletions of ATM in mice result in infertility and chromosomal fragmentation during meiosis as well as other features of ataxia-telangiectasia (42). It has been shown recently that ATRp and ATMp localize at complementary sites on pairing forks in mitotic prophase, ATRp on the unsynapsed axes and ATMp on the synapsed side (43). These observations are not only pointers to human autosomal genes with effects on spermatogenesis, but also will enhance understanding of the biochemistry of meiosis.

LESSONS FOR HUMAN SPERMATOGENESIS

What do defects in these mouse autosomal genes tell us about human infertility? First, they identify defects in single genes that can have catastrophic results for spermatogenesis while the rest of the animal is unaffected. This male sterile-specific effect is phenotypically similar to idiopathic infertility in humans and suggests that here too, single-gene mutations might be important. How precisely these mouse mutations model human infertility will become clear only as the molecular basis of human infertility is elucidated. The experiments in mice have identified genes as possible targets for mutagenesis screens in populations of infertile men, which is a conceivable first step in such an analysis.

The finding of an increasing number of genes in which defects cause infertility in the mouse strongly suggests that a similar number of genes might be needed for fertility in humans. This finding suggests that idiopathic infertility might have a number of disparate genetic causes. However, a potential unifying observation is that the two candidate Y-encoded AZF genes in humans are putative RNA-binding proteins and so are likely to be involved in regulating gene expression. The autosomal genes are potential target transcripts both for regulation by these RNA-binding proteins and for defective regulation in men with Y-chromosome deletions.

A particularly attractive candidate for such a scenario is the CREM transcript, which has a complex pattern of RNA

processing. CREM is a transcription factor that can either up-regulate or down-regulate transcription of specific genes depending on the splicing of its mRNA. Disruptions of the gene in mice result in a block in formation of the haploid stages of spermatogenesis.

GENETIC IMPLICATIONS OF ICSI

The critical difference between genetic and other causes of infertility is that genetic defects giving rise to abnormal spermatogenesis of a type that can be surmounted by ICSI will be transmitted to the children produced. In the case of Y-chromosome deletions, this is only a concern for male offspring. At worst, these children would be expected to experience fertility problems similar to those of their fathers.

Currently, we cannot be sure of the absolute nature of the link between Y-chromosome deletions and spermatogenic defects. Studies are needed to determine the exact frequency of various Y microdeletions in men who are participating in ICSI and a corresponding determination of deletion frequencies in their children. All centers with an ICSI program should be encouraged to participate in such studies. The information acquired from multiple centers would rapidly reveal the extent of the problem and the relevance of screening, and clinical practice could be modified accordingly. If a causative link is proved, then tests can be refined rapidly to focus on the relevant gene(s) within the deletion.

General screening for mutations in autosomal genes implicated in infertility alone seems to be unwarranted at this time because no single gene is likely to contribute to a large fraction of cases. In rare families in whom a history of infertility is consistent with a genetic basis, we may soon have a set of genes for which screens may be justified. There are genes that affect fertility in addition to other functions. For example, the cystic fibrosis transmembrane regulator (CFTR) gene is associated with congenital bilateral absence of the vas deferens and, in this case, the frequency of mutations justifies screening.

CONCLUSIONS

The products of many genes are essential for spermatogenesis, but a smaller number have effects exclusively in spermatogenesis. Observations in humans have shown us some of these genes, and experiments in mice have revealed others. The striking difference is the contribution that mutations, including deletions, may make to human male infertility. This is particularly true in the case of Y-chromosome microdeletions in humans and spermatogenesis, although more detailed information on epidemiology is needed.

Because some mutations affect only male fertility, not female, and arise only when homozygous, these mutations could be propagated in females as homozygotes and in males as heterozygotes. Defects in spermatogenesis can be caused by effects in both germ cells and in somatic cells in the testis. The identification of these genes and the potential role of their products will make an important contribution to our understanding of the biology of spermatogenesis.

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