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REVIEW ARTICLE

INSIGHTS INTO THE MOLECULAR BASIS OF MALE INFERTILITY

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Abstract : The study of the genetic basis of human male infertility is complicated by genetic heterogeneity and because linkage analysis studies are difficult. The study has been limited so far to the analysis of genes located on the Y chromosome. Several genes and gene families have been discovered and mutation analysis of these candidate genes in infertile patients is ongoing. In recent years, several mouse models with impaired spermatogenesis or fertility have also been analysed, expanding our knowledge about the molecular basis of spermatogenesis and male fertility.

Key words : genetics, germ cell, male infertility, spermatogenesis, sperm-egg interaction, Y-chromosome

INTRODUCTION

Male fertility is complex and depends upon endocrine/paracrine regulatory mechanism and morphogenetic processes occuring during testicular development, spermatogenesis and spermiogenesis. Male infertility affects approximately 2–7% of couples around the world. For about 30% of men, infertility has a genetic origin (1). The genetic causes affecting male fertility can therefore be mutations of genes that depress steroid production (2), mutations of genes that affect specifically germ cell development or sperm function, and mutations in genes that affect several organs including testis (e.g. cystic fibrosis gene, 3). Stress or environmental factors, such as infection and pollution, have also been described or suggested to cause male infertility (4).

The present review will focus on our understanding of genes required for normal spermatogenesis and male fertility. So far, very few of these genes have been identified in humans, but several mouse models with impaired spermatogenesis and fertility have been described (5–7). In the first part of this review we will focus on the role of the human Y chromosome in male infertility. In the

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second part we will describe genes important for male gametogenesis and fertility identified through the analysis of mouse mutants.

Y chromosome and male fertility

Until now, the search for genes involved in human spermatogenesis and fertility has been focused on the Y chromosome. The involvement of the human Y chromosome in male infertility was first suggested in 1976 by Tiepolo et al (8). In some infertile men, Tiepolo and colleagues detected cytogenetic deletions of the long arm of the Y chromosome. Later, extensive molecular studies have defined three deletion intervals (*AZFa*, *AZFb* and *AZFc* for azoospermia factors a, b and c) (Figure 1, 9–13). Deletions of the *AZFa* region are associated with azoospermia manifested by Sertoli cell only syndrome (SCOS) or sometimes with oligozoospermia (10, 14, 15). Deletions of the *AZFb* region are associated with azoospermia (SCOS, meiotic and maturation arrest), oligozoospermia and normozoospermia (10, 11, 16), whereas deletions of the *AZFc* interval are associated with azoospermia (similar causes to that observed with *AZFb* deletions) and severe to mild oligozoospermia (10, 17, 18). Detailed molecular analysis of the Y chromosome (19–21) has enabled the identification of several candidate genes within the *AZF* intervals.

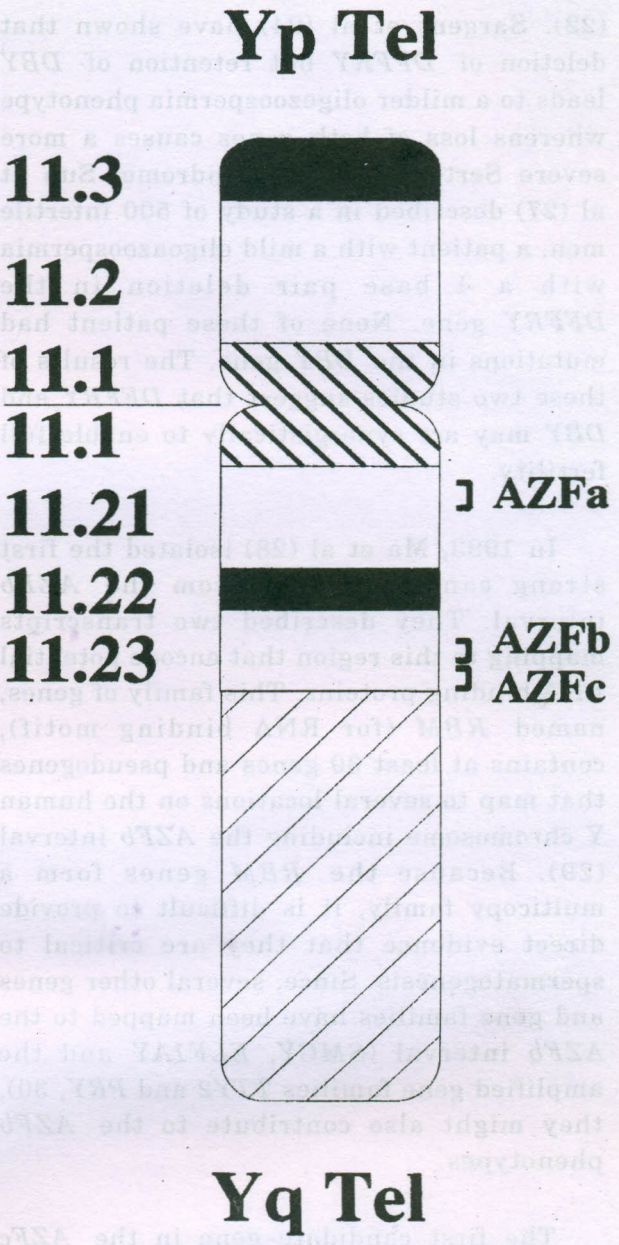

Fig. 1: Y deletion intervals associated with defective spermatogenesis.

Two genes, *DFFRY* and *DBY* have been identified within the *AZFa* interval (22, 23–25). *DFFRY* (Drosophila Fat Facets Related) is expressed ubiquitously and encodes a ubiquitin specific protease closely related to the Drosophila *faf* gene (26). *DBY* (DEAD box on the Y) encodes a protein containing the DEAD box motif found in RNA helicases

(22). Sargent et al (24) have shown that deletion of *DFFRY* but retention of *DBY* leads to a milder oligozoospermia phenotype whereas loss of both genes causes a more severe Sertoli cell only syndrome. Sun et al (27) described in a study of 500 infertile men, a patient with a mild oligoazoospermia with a 4 base pair deletion in the *DFFRY* gene. None of these patient had mutations in the *DBY* gene. The results of these two studies suggest that *DFFRY* and *DBY* may act synergistically to enable full fertility.

In 1993, Ma et al (28) isolated the first strong candidate gene from the *AZFb* interval. They described two transcripts mapping to this region that encode potential RNA binding proteins. This family of genes, named *RBM* (for RNA binding motif), contains at least 30 genes and pseudogenes that map to several locations on the human Y chromosome including the *AZFb* interval (29). Because the *RBM* genes form a multicopy family, it is difficult to provide direct evidence that they are critical to spermatogenesis. Since, several other genes and gene families have been mapped to the *AZFb* interval (*SMCY, ELF1AY* and the amplified gene families *TTY2* and *PRY*, 30), they might also contribute to the *AZFb* phenotypes.

The first candidate gene in the *AZFc* interval was isolated by Reijo et al (18) in 1995. They identified a gene, named *DAZ* (for deleted in azoospermia) that encodes an *RNA* binding protein. Analysis of the *DAZ* locus in the *AZFc* region has demonstrated that, like *RBM*, *DAZ* arises from an Y-linked multicopy family (31, 32). *DAZ* is expressed specifically in testis (33) and is related to a Drosophila fertility gene named *boule,* which when mutated causes azoospermia (34).

An autosomal homologue of *DAZ* has been described in both human and mouse but no *DAZ* gene has been found on the mouse Y chromosome. The human gene (named *DAZLA*), located in 3p24, is expressed specifically in the testis and at lower levels in ovary (31, 35–37). The mouse gene (*Dazla*) has been mapped to chromosome 17 and is also expressed in both male germ cells and the female gonad (38, 39). Disruption of the mouse *Dazla* gene leads to a complete loss of germ cells, demonstrating the critical role of this gene in both, male and female gametogenesis (40). *DAZ* is an attractive candidate for the *AZFc* phenotype, but the existence of a gene family exacerbates efforts to search for mutations. Other genes have been mapped to the *AZFc* interval (*BPY2*, *PRY* and *CDY*, 22). Stuppia et al (41) have reported deletions of the *AZFc* region causing infertility, that do not remove *DAZ* genes, suggesting that other loci in the *AZFc* interval may contribute to fertility in addition to *DAZ* or that modifying genes in the genetic background of an individual may be able to compensate.

Genetic research into male infertility, in the last 7 years, has resulted in the isolation of several genes or gene families on the human Y chromosome. Studies with more infertile patients are required to resolve the implication of these candidate genes mapped in the different *AZF* intervals.

Mouse models

Identification of genes specifically involved in human spermatogenesis and fertility has been limited so far. Analysis of natural or genetically engineered mouse mutants has permitted the identification of more loci and genes critical for spermatogenesis and male fertility. More than 50 natural mouse mutants with a defective reproductive system have been described (see list on the Mouse Genome Database, http://www.informatics.jax.org). Some mutants display a complete lack of mature germ cells (e.g. mouse *gcd*, germ cell deficient) whereas others present abnormal spermatozoa (e.g. mutant *azh*, abnormal spermatozoa head shape). For most of these mutations the locus has been precisely mapped on a chromosome but the genes remain unknown.

Using overexpression of transgenes or gene inactivation (by homologous recombination or gene trap mutagenesis), more infertile mice have been generated and they can be used to study the function of particular genes in mouse male gametogenesis and fertility. Table I presents a list of genetically engineered mouse mutations affecting male reproduction. This table has been divided into mutations that have a specific testicular function and those that have an additional impact on other biological functions. We will briefly describe two mouse mutants with impaired spermatogenesis or fertility respectively.

TABLE I: Genetically engineered mouse mutants with impaired spermatogenesis or fertility.

Genes	*Function*	*Reference*
Genes involved specifically in spermatogenesis or fertility		
aromatase	steroid metabolism	45
Bcl2	anti apoptosis regulator	46
Bcl-w	anti apoptosis regulator	47, 48
Bcl-x	anti apoptosis regulator	49
Bmp8B	bone morphogenetic protein	50
calmegin	chaperone	51
Casein kinase II a' catalytic subunit	regulation of metabolism	52
CREM	transcriptional regulator	53, 54
c-ros	tyrosine kinase receptor	55
cyclin A1	cell cycle regulator	42
cyritestin	sperm protein	56
E2F-1	transcription factor	57
Egr4	transcription factor	58
E-MAP-115	microtubule-associated protein	59
fertilin β	sperm surface protein	43
Hr6B	ubiquitin conjugating enzyme, DNA repair	60
HSF1	heat shock transcription factor	61
Hsp 70.2	heat shock protein	62
Pc4	processing of prohormones	63

Pplc γ	protein phosphatase	64
Protamine 1	chromosome condensation	65
$P2X_1$ receptor	ATP channel receptor	66
SCP3	chromosome pairing	67
Tarbp2	RNA binding protein	68
THEG	protein assembly	69
transition nuclear protein 1	histone replacement/chromosome condensation	70*
Genes involved in spermatogenesis or fertility with other effects		
A-myb	DNA binding protein	71
ACE (angiotensin-converting enzyme)	blood pressure regulation	72
Apaf-1	pro apoptosis regulator	73
apolipoprotein	lipoprotein metabolism	74, 75
Atm	mutated in Ataxia-telangiectasia	76, 77
Basigin	cell surface protein	78
Bax	pro apoptotic regulator	79
Ca2+/calmodulin-dependent kinase IV	transcriptional regulator	80
Cyclin-dependent kinase 4	cell cycle regulator	81
Dazla	RNA binding protein	40
Dmc1	DNA recombination protein	82, 83
Estrogen alpha receptor	steroid metabolism	84
Hoxa10	homeotic gene	85
HSL (hormone-sensitive lipase)	obesity and steroidogenesis	86
Igf1	insulin-like growth factor 1	87
Jun D	transcription factor	88
Kit receptor tyrosine kinase	cell signaling	89
Mlh1	DNA mismatch repair enzyme	90, 91
Msh4	DNA mismatch repair enzyme	92
Msh5	DNA mismatch repair enzyme	93
Na^+–K^+–2Cl cotransporter	ion transporter	94
Nfia	nuclear factor	95
Nhlh2	transcription factor	96
Nek1	polycystic kidney disease	97
OSP	oligodendrocyte-specific protein	98
PDGF-A	platelet-derived growth factor	99
Pms 2	DNA mismatch repair enzyme	100
Prolactin receptor	lactation, reproduction	101
RAR a receptor	retionoic acid receptor	102
RAR γ receptor	retinoic acid receptor	103
Rho GDIa	Rho GDP dissociation inhibitor	104
RXR β receptor	retinoic acid receptor	105
Telomerase	maintenance of chromosome	106
TLS (translocated in liposarcoma)	RNA binding protein	107
Zfx	zinc-finger transcription factor	108

*Infertility only in approximately 60% of homozygote male mutants.

In 1998, Liu and colleagues described a mutation in cyclin A1, a cell cycle protein gene, that causes male infertility (42). Mutant mice were generated by inactivating the mouse *Ccnal* gene by homologous recombination in embryonic stem cells. *Ccnal* appears to be essential for spermatogenesis and without dramatic effects in other tissues. Female mice and heterozygote males were fertile whereas *Ccnal* -/- males were infertile. Testes from homozygote mutants were smaller and spermatogenesis was completely disrupted. Analysis of the homozygote *Ccnal*-deficient mice showed that the early testicular development was normal, but spermatogenesis was arrested at late meiotic prophase. A more detailed characterization of this meiotic arrest, shows an increase of spermatocyte apoptosis.

The phenotype of *Ccnal* -/- mice establishes the essential role of cyclin A1 for the entry of male germ cells into the first meiotic division. The lack of observable defects during oogenesis indicates different mechanisms of regulation of meiosis between the two sexes. Cyclin A1 represents a novel class of cyclin that performs an essential male germ cell-specific function and can be a good candidate gene for human male infertility with meiotic arrest.

In 1998, Cho et al generated a mouse model in which the gene encoding fertilin β has been inactivated (43). Fertilin β along with fertilin α forms a sperm surface protein located on the surface of acrosome-reacted sperm head, which interacts with integrin receptors on the oocyte.

Homozygous mice deficient in the fertilin β gene were infertile. Fertilin β -/- sperm were normal and underwent normal acrosome reaction. *In vitro* sperm-egg adhesion assays showed that the binding and the fusion of the mutant sperm to the egg membrane were reduced. Fertilin β -/- sperm were also unable to adhere to the zona pellucida and were rarely found in the oviduct. These results show a direct role of fertilin β in sperm-egg membrane interaction. Recent results suggest that fertilin β and α6β1 integrin interact, via a cooperation between α6β1 integrin and CD9 (present in the oviduct and on the egg, 44). Such cooperation may assist sperm passage into the oviduct as well as sperm-egg interactions.

In humans, male infertility may occur in the absence of obvious sperm defects and fertilin β may be a candidate gene. Other genes involved in sperm-egg interaction may also be promising candidates for fertility defects. Analysis of mouse mutants has expanded our knowledge about the molecular basis of spermatogenesis and male fertility. However, for most of the human cases of infertility, the genetic defects remain unknown. Animal models of male infertility are valuable tools to identify candidate genes for particular catagories of human male infertility.

CONCLUSION

With the sequencing of the human genome near completion, the discovery of all genes is expected. New genes that play a role in spermatogenesis and male fertility will be uncovered. This will lead to better diagnostics and improved treatments of male infertility.

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