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<Review>

Genetic Causes in Male Infertility of Human

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남성 불임의 유전성 요인

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ABSTRACT : It is believed that genetic defects make an important contribution to male infertility. Since spermatogenesis is such a complex process, it seems inevitable that many genes are involved in controlling the entire development of germ cells. Genes for infertility, however, are considered to be only those which are defected in the reproduction ability, but normal in other functions. Microdeletions of the Y chromosome have been observed frequently in infertile males. At least two genes, *RBM* and *DAZ*, are known to present in the loci where microdeletions occur frequently. A number of autosomal genes were also considered as candidates of infertility genes, based on phenotypes of knockout mice that were deficient of these genes.

Key words : Male infertility, Y chromosome, Knockout mouse, Genetic disease.

요 약 : 남성 불임에 있어서 유전적 결함은 중요한 요인 중 하나이다. 정자 형성은 매우 복잡한 과정을 거치므로 생식세포의 완전한 발달을 조절하는데 많은 유전자들이 관여할 개연성이 높다. 이들 유전자 중에서 불임 유발 유전자는 다른 기능은 정상이되 생식에만 제한적으로 영향을 미치는 유전자들로 정의될 수 있다. 불임 남성에게서 Y 염색체의 미세결실(microdeletion)이 자주 관찰되었고, *RBM*과 *DAZ*라는 유전자가 그 위치에 존재함이 알려졌다. 또한 상 염색체에 존재하는 유전자를 인위적으로 변이시킨 생쥐들 가운데 표현형이 웅성불임으로만 국한하여 나타나는 경우가 있는데, 이런 유전자들도 남성 불임 유전자의 후보로 간주할 수 있으며, 실제로 이 유전자들의 변이에 말미암은 남성 불임 환자도 적지 않을 것으로 사료된다.

INTRODUCTION

Approximately 15% of all couples attempting to establish their first pregnancy meet with failure (Mosher, 1987). Two to seven percent of couples remain childless until the end of their reproduction life (Spira, 1987). It was estimated that male factor infertility accounts for about half the cases of couple infertility. These numbers reveal how abundant male infertility cases are in real life.

Etiology of male infertility can be classified into infections, structural abnormalities in reproductive organs, psychological problems and genetic defects (Kim & Lipschultz, 1997). The proportions of causes on male infertility are not clearly understood yet, although the concern that male infertility rates may be on the rise in human (Carlsen et al., 1992), possibly as a result of environmental toxins such as analogues of sex hormones. In the case of genetic causes, it was predicted that genetic defects make an important contribution to cases of idiopathic infertility. In fact, the origin of reduced testicular function is unknown in more than 60% of cases, but they may have an unidentified genetic anomaly (Krausz & McElreavey, 1999).

Spermatogenesis is an elaborate process involving both cell division and differentiation, and cell-cell interactions. A large number of genes participate in the process. In principle, genes that are involved in development of male germ cells can be responsible for male infertility. In practice, however, genes for male infertility have been limited to those that generate phenotypes restricted mostly at the reproductive activity. Defects that are detected in these individuals are restricted to spermatogenesis and these men

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are usually otherwise healthy, suggesting the genes involved must either be only expressed in spermatogenesis, or be functionally required only for spermatogenesis (Elliott & Cooke, 1997). For example, testicular atrophy is recognized as a feature of myotonic muscular dystrophy (Harper, 1989). However, the patients cannot afford to complain of male infertility that is accompanied with the serious disease. Therefore, expression of genes for male infertility is frequently found to be limited within reproductive organs, particularly within the germ cells.

It was reported that most of infertility genes are expressed within the germ cells and play critical roles in their development. In some cases, however, there were infertility genes that play roles in other cell types, but not in germ cells within the reproductive organs. The testis contains a few somatic cell types. Sertoli cells are nurse cells found within the seminiferous tubules. Myoid cells are contractile cells found on the periphery of the tubules, and Leydig cells are found in the intertubular space and secrete testosterone. It is possible that defects in such somatic cells can cause interruptions in spermatogenesis. The best example may be the *Desert Hedgehog* (*Dhh*) gene that encodes a signaling molecule. *Dhh* is expressed specifically in Sertoli cells but not in germ cells within the testis. *Dhh* gene targeting experiments revealed that male mice homozygous for a *Dhh*-null mutation are viable but infertile, owing to a complete absence of mature sperms (Bitgood et al., 1996). These results demonstrated that Sertoli cell-germ cell interactions are critical for sperm development.

It is still far from completion in understanding genetic cause of male infertility (Okabe et al., 1998). However, recent progress in Y chromosome studies has accumulated a great amount of information on genes for development of male germ cells. At the same time, male sterility has been observed in a number of knockout mouse studies as predicted or, in many times, unexpected. Such genes can be candidates for the male infertility genes. In this review, we will discuss the candidate genes for male infertility. We will focus on genetic defects which caused problems in male fertility but healthy otherwise.

Y CHROMOSOME

1. Structure of the Y chromosome

The Y chromosome is the smallest one among the human chromosomes, and consists of merely 2% of total genome. Studies of human pedigrees have identified many traits exhibiting autosomal or X-linked inheritance but no convincing cases of Y-linked inheritance (Okabe et al., 1998). The Y chromosome has been central to research on male infertility because of its haploid nature and dispensability. Reports of XO females and XXY males established the existence of a sex-determining gene on the human Y chromosome.

Y chromosome can be divided into two regions: pseudoautosomal regions (PARs) that share homology with X chromosome and a non-recombining region in Y chromosome (NRY) (Ellis, 1998). PARs are located at both tips of the Y chromosome and mediate pairing with their homologous regions on the X chromosome, where they form a crossing-over. A failure either to pair or to recombine results in meiotic arrest (Rappold, 1993; Burgoyne & Mahadevaiah, 1993; Ashley et al., 1994). In NRY, two dozens of genes have been identified.

The genes in NRY can be sorted into two discrete classes (Elliott & Cooke, 1997). The first group, of five novel NRY genes, has several shared features. Each gene has a homologue on the X chromosome encoding a very similar but non-identical protein isoform; every gene is expressed in a wide range of human tissues; and each gene appears to exist in a single copy on the NRY. Most of the X-homologous genes appear to be involved in cellular housekeeping, as suggested by their ubiquitous expression and by the functions of their encoded proteins. *TB4Y* encodes a Y isoform of thymosin B4, which functions in actin sequestration. *EIF1AY* encodes a Y isoform of eIF-1A, an essential translation initiation factor. *RPS4Y* encodes a Y isoform of an essential ribosomal protein.

The others constitute the second group and share quite different traits. They appear to be expressed specifically in the testis. They also seem to exist in multiple copies on the NRY. In contrast with single-copy, X-homologous house-

keeping, genes the multi-copy NRY gene families appear to encode proteins whose functions are limited to male germ cell development. Genes in this group are under investigation for possible connections with male infertility.

2. Deletion mutations on Y chromosome

Although the functions of the various loci/genes on the Y chromosome are not clearly known yet, many reports support the idea that microdeletions of Y chromosome play a causal role in male infertility (Simoni et al., 1998). Microdeletions of the long arm of the human Y chromosome are associated with spermatogenic failure and have been used to define three regions of Yq (the azoospermic-factor loci *AZFa*, *AZFb*, and *AZFc*) that are recurrently deleted in infertile males (Vogt et al., 1996). About 10~15% of azoospermic and about 5~10% of severely oligospermic men have Yq microdeletions (Krausz & McElreavey, 1999). Microdeletions were found almost exclusively in infertile men with azoospermia or with sperm concentration of $<5\times 10^6$/ml (Simoni et al., 1998). Furthermore, overall combined odds ratio of all studies shows that azoospermic patients have a significantly higher frequency of microdeletions compared with oligospermic men (Simoni et al., 1998).

There is no clear-cut association between location of deletion and phenotype. However, it seems that men with deletions involving the more proximal parts (*AZFa* and/or *AZFb*) are generally azoospermic (Silber et al., 1998). To date only one patient with a deletion involving only the *AZFa* region and severe oligospermia has been described (Qureshi et al., 1996). Nevertheless, it is clear that deletions in *AZFc* are sometimes compatible with some degree of ongoing spermatogenesis (Simoni et al., 1998). These data suggest that even large deletions in the *AZFc* region are compatible with a certain degree of fertility and indicate a possible evolution of oligospermia to azoospermia, at least in some cases (Vogt et al., 1995).

3. *RBM* family genes in the Y chromosome

With collections of deleted Y chromosomes from infertile males, the *AZF* locus has been mapped within cytological band Yq11.23 (Teipolo & Zuffadi, 1976). Extensive searches began for the *AZF* genes with patients who were azoo- or severly oligospermic, and eventually identified the *RBM* gene family (Ma et al., 1993).

The *RBM* gene family consists of several subfamilies and approximately 30 genes and pseudogenes, is found on both arms of the Y chromosome, but is predominantly clustered within the *AZF* locus in Yq (Elliott & Cooke, 1997). *RBM* expression was studied by use of RT-PCR of *RBM* transcripts and by characterizing dozens of *RBM* cDNA clones (Chai et al., 1997). Two *RBM* cDNA clones, *MK5* and *MK29*, that encode an RNP motif near the N-terminus and four tandem repeats of a 37-residue peptide (the SRGY box) in the middle of the polypeptide chain have been described (Ma et al., 1993). Whereas the RT-PCR experiments suggested the existence of at least six *RBM* subfamilies (*RBM*I to *RBM*VI), the cDNA cloning experiments indicated that only *RBMI* is actively transcribed due to some silent base substitutions (Chai et al., 1997). Northern blot hybridization analyses using *MK5* probe, a *RBMI* family member cDNA, revealed that the *RBM* is expressed exclusively in the testis among adult tissues examined (Chandley, 1998). The conservation of Y-linked *RBM* genes among mammals implies an importance of their functions in male fertility (Ma et al., 1993).

A specific antiserum was used to examine expression pattern of *RBM* in both the adult and fetal testes (Elliott et al., 1996;1997). In adult human testes, only germ cells were stained with the antiserum. Strong nuclear staining was observed in both type A and B spermatogonia, spermatocytes, and round spermatids. No *RBM* protein is detectable in elongating spermatids.

RBM encodes a protein with a single RNA recognition motif, a sequence of amino acids shown to bind RNA. *RBM* is closely related to the autosomal hnRNPG (Le Coniat et al., 1992), and both are members of the hnRNP family, which are associated with nuclear polyadenylated RNA and are involved in pre-mRNA packaging, transport to the cytoplasm and splicing (Weighardt et al., 1996). Therefore, it is tempting to speculate that, like hnRNPA1p, *RBM* may play a role in regulating splicing (Caceres et al., 1994). Many testis-specific alternative splicing events have been described and it is possible that *RBM*-dependent splicing events could be essential for spermatogenesis.

Even if there is a strong correlation between microdeletion on the *RBM* genes and male infertility, we still are not able to understand the mechanisms involved. The role for *RBM* in spermatogenesis is harder to demonstrate for at least three reasons. First, deletions in infertile males generally involve some but not all *RBM* genes because there are a large number of the *RBM* genes found in several regions of the Y chromosome. Second, assessment of gene function by the generation of knock-out mice may be difficult due to multiple copies of *Rbm* on the mouse Y chromosome (Elliott et al., 1996). Finally, the presence of multiple functional *RBM* genes, some encode the same protein isoforms (Chai et al., 1997).

4. *DAZ*: Male infertility gene in the *AZFc* region

DAZ (Deleted in Azoospermia) is a candidate gene for the Y chromosome azoospermia factor. The human *DAZ* gene is present in multiple copies (at least two or three copies) on the *AZFc* region of the Y chromosome. Comparative studies of fertile and infertile men indicate that mutations in *DAZ* are extremely frequent, occurring at a frequency of at least 1 in 8,000 men (Reijo et al., 1995; 1996). Deletions of the *DAZ* genes are found frequently in azoospermic or in severe oligospermic males (Vereb et al., 1997).

DAZ encodes a protein with an RNA-binding motif. A structural homologue of the human *DAZ* was isolated from the mouse genome. Interestingly, *Dazla* (*DAZ-like autosomal*), the mouse gene, is localized to chromosome 17 (Cooke et al., 1996). It turned out that at least four non-primate mammals (mice, rabbits, dogs, cattle) appear to carry a single, autosomal homolgue of *DAZ*. Nucleotide similarity between Y-linked human *DAZ* and autosomal mouse *Dazla* (85% identity in the coding region) suggests that they were derived from a common ancestral gene during mammalian evolution (Reijo et al., 1996).

Disruption of the mouse *Dazla* gene leads to three fold reduction of testis mass, loss of germ cells and complete absence of gamete production, demonstrating that *Dazla* is essential for the differentiation, development and survival of germ cells (Ruggiu, 1997). *Dazla*$^{+/-}$ males showed high level of abnormal sperm (no sperm in *Dazla*$^{-/-}$ mice) and fewer tubules than those of wild type mice, indicating that there is a quantitative requirement (Ruggiu, 1997). Thus, it was thought that *DAZ* could be a candidate gene for normal sperm production of human, as anticipated by the function of the mouse *Dazla* protein.

A fly homologue of *DAZ*, Boule, was cloned and characterized (Eberhart et al., 1996). Sequence comparisons indicated that the putative RNA-binding domain of the Boule protein is most similar (42% identity) to that of the human *DAZ* protein. Boule also has considerable sequence similarity (33% identity) to a second region of *DAZ*, so called the *DAZ* repeat. Boule expression appeared testis -specific. Moreover, loss-of-function mutations of Boule resulted in azoospermia: meiotic divisions were blocked, although limited spermatid differentiation occurred (Eberhart et al., 1996). These results support the idea that *DAZ* is a human *AZF*, and indicate that Boule and *DAZ* have an essential meiotic function in fly and human spermatogenesis.

CANDIDATE GENES IN AUTOSOMAL CHROMOSOMES

Spermatogenesis is such a complex process that it seems inevitable that many genes are involved in controlling the entire development of germ cells. Although search for male infertility genes has largely focused on the Y chromosome, there is evidence of involvement of autosomal genes. In fact, families with multiple infertile males have previously been reported (Lilford et al., 1994). This study predicted that autosomal modes of inheritance could account for 60% of subfertility in men (Lilford et al., 1994).

Male infertility has also attracted a great deal of recent attention from geneticists and molecular and cell biologists, who have created targeted disruptions of genes in the mouse. Knockout technology has made the mouse a favored experimental organism for studies of gametogenesis. What do defects in these mouse autosomal genes tell us about human infertility? There were defects in single-genes that can have catastrophic results for spermatogenesis while the rest of the animal is unaffected (Cooke et al., 1998). This male sterile-specific effect is phenotypically similar to

idiopathic infertility in humans. Genes identified in the knockout experiments may be possible targets for the mutation screens in populations of infertile men. The finding of an increasing number of genes whose defects cause infertility in the mouse strongly suggests that a similar number of genes might be needed for fertility in humans.

1. *A-Myb*

The *Myb* gene family, which encodes transcription factors, consists of three members, named A-, B-, and *C-Myb*. In adult mice, *A-Myb* is expressed predominantly in male germ cells (Mettus et al., 1994). The first observable feature of the *A-Myb*$^{-/-}$ phenotype was the small size of the mice, apparent during the first few weeks of life (Toscani et al., 1997). The size and weight of the testes from A-*Myb*$^{-/-}$ mice were about 25% of those of their littermates and there was a slight reduction in the size of the seminiferous tubules. Spermatogonia and pre-leptotene spermatocytes were normal, whereas most pachytene spermatocytes showed different degrees of degeneration, from early nuclear changes to advanced stages of cellular breakdown. Most notably, there was a complete absence of post-meiotic cells such as spermatids or spermatozoa, indicating that spermatogenesis came to an abrupt halt at the pachytene stage of meiosis. Sperm counts showed that there was a complete absence of spermatozoa in *A-Myb*$^{-/-}$ testes. Thus, it was predicted that men who have mutations in the A-*Myb* gene cause male infertility due to the arrest of spermatogenesis at pachytene stage of meiosis.

2. *PMS2*

PMS2 is a mammalian homologue of E. coli mutL that plays a central role in the correction of replication errors. It was proposed that *PMS2* may be involved in DNA mismatch repair in mammalian cells. *PMS*-deficient mice showed no embryonic or neonatal lethality (Baker et al., 1995). One prominent aspect of the *PMS*-deficient phenotype is male infertility. Abnormality was detected in chromosome synapsis during meiotic prophase spermatocytes. This phenotype may be linked to defects in mismatch repair, genetic recombination, and initiation of chromosome synapsis during meiosis. *PMS2* can be a candidate infertile gene in azoospermic or oligospermic patients.

3. *Hsp70-2*

The 70-kDa heat shock proteins (HSPs) are chaperones which assist the folding, assembly and disassembly of other proteins (Georgopoulos & Welch, 1993). Hsp70-2 is a unique member of the mouse Hsp70 family expressed during prophase of meiosis I in male germ cells (Allen et al., 1988). Hsp70-2 gene expression is regulated developmentally in mouse testis (Dix et al., 1996). Hsp70-2 is a lateral component of spermatocyte synaptonemal complexes (SCs) from zygotene through diplotene, suggesting that it could be significant for SC formation (Allen et al., 1996). Hsp70-2$^{-/-}$ mice seemed to arrest the development at the end of the pachytene stage and to become apoptotic (Dix et al., 1997). In Hsp70-2$^{-/-}$ mice, the formation of SCs was normal with no evident increased asynapsis or mispairing. However, desynapsing SCs, which is characteristic of the spermatocytes, was not identified (Dix et al., 1997). Hsp70-2$^{-/-}$ mice showed the Cdc2/cyclin B1 complex failed to assemble and Cdc2 kinase activity was not present in the testis. It is likely that disruption of Cdc2/cyclin B1 complex assembly is also one cause of failure for Hsp70-2$^{-/-}$ spermatocytes to complete meiosis I (Zhu et al., 1997). Therefore, Hsp70-2 is a candidate gene for male infertility in a sense that Hsp70-2 regulates desynapsis of SCs in spermatocytes and meiotic division in a normal spermatogenesis.

4. *MLH1*

MLH1 is a DNA mismatch repair gene. *MLH1* forms the complex with PMS1 (Li & Modrich, 1995), and interacts with MSH2 which recognizes DNA mismatch (Prolla et al., 1994b). *MLH1*$^{-/-}$ mice were viable but were prone to have replication-error phenotype due to deficiency in mismatch repair activity. In spite of normal mating behavior, male mice showed a half size of testes compared to normal males, slight reduction in the size of seminiferous tubules, and no detectable mature sperm. Spermatogenesis enters meiotic prophase. Most tubules contained a basal layer of spermatogonia and Sertoli cells along with a few layers of

primary spermatocytes at the leptotene to pachytene stage, but spermatocytes were arrested at pachytene stage (meiosis I arrest), no spermatocytes beyond this stage were observed (Edelmann et al., 1996). Female mice showed normal mating behavior and production of oocytes, but infertilty due to a failure of development beyond the single-cell stage of eggs (Edelmann et al., 1996). These results suggested that *MLH1* is required for normal meiosis of germ cells in both sexes. Therefore, *MLH1* is believed to result in human infertility of both sexes on the basis that *MLH1* is required for normal process of meiosis.

5. *Cyclic AMP-response element modulator (CREM)*

The cyclic AMP-response element modulator (CREM) gene encodes a family of transcriptional regulators that bind to promoter sequences activated by increased intracellular cAMP levels. Both activators and repressors are generated by alternative splicing and by alternative translational initiation. It was reported that expression of the CREM activator protein is restricted to postmeiotic germ cells (Delmas et al., 1993). Since the rise in CREM tau protein coincides with the transcriptional activation of several genes, Sassone-Corsi (1997) proposed that CREM may have a role of the master switch in postmeiotic male germ cell development.

Comparison of the homozygous *CREM*-deficient mice with their normal and heterozygous littermates indicated no macroscopic physical aberrations or reduction in body weight (Nantel et al., 1996). However, analysis of the seminiferous epithelium in mutant male mice reveals postmeiotic arrest at the first step of spermiogenesis (Nantel et al., 1996). Late spermatids were completely absent, and there is a significant increase in apoptotic germ cells. The complete lack of spermatozoa in the mutant mice may be reminiscent of human infertility cases.

6. *HR6B*: a ubiquitin-conjugating DNA repair enzyme

Two closely related human homologues of *RAD6* were designated h*HR6A* and h*HR6B* (for human Homologs of *RAD6* ; Koken et al., 1991). *HR6B* is implicated in postreplication repair and damage-induced mutagenesis (Lawrence, 1994). *HR6B*-deficient mice showed viable and normal phenotype, presumably due to functional redundancy with *HR6A*. But they were infertile although heterozygous male and homozygous female were fertile (Roest et al., 1996). Histological evaluation of the testis and epididymis of adult $HR6B^{-/-}$ male mice showed a strong derailment of spermatogenesis. A nearly total absence of all germ cell types was found in 10%-20% of $HR6B^{-/-}$ male mice, but in most knockout males spermatogenesis went on with only low numbers of predominantly abnormal spermatozoa (Roest et al., 1996). It was shown that *HR6B* can ubiquitinate histones *in vitro* and is expressed especially in round spermatids. Based on these results, it was proposed that *HR6B* may replace somatic and testis-specific histones with transition proteins, subsequently protamines. Thus, *HR6B* may be a candidate gene for male infertility, in that *HR6B* is required for the compaction of chromatin in spermatogenesis.

7. *Phosphatase 1cγ (PP1cγ)*

Protein phosphatases can be classified into four main types by biochemical criteria: Protein phosphatase type 1 (PP1), type 2A (PP2A), type 2B (PP2B), and type 2C (PP2C ; Wera and Hemmings, 1995). PP1 is involved in diverse cellular activities, ranging from glycogen metabolism to chromatin structure modification, mitosis, and meiosis. The holoenzymes are composed of catalytic subunits (PP1c) and one or more regulatory subunits. Many eukaryotes possess multiple genes encoding PP1c isoforms (Ohkura et al., 1989). The *PP1cγ* isoforms are generated by differential splicing of the primary transcript of the *PP1cγ* gene. In rodents, one of these isoforms, *PP1cγ2*, appears to be expressed predominantly in testes.

The *PP1cγ* (both *PP1cγ1* and *PP1cγ2*) null mutation is not lethal at any stage of embryogenesis. Mutant males produced relatively normal levels of testosterone and were able to plug receptive females. They predominantly had round spermatids, with only the occasional sperm visible, suggesting spermiogenesis was impaired (Varmuza et al., 1999). The round spermatid population showed distinctive abnormalities that could be explained by meiotic failure. Occasional meiotic spindles were abnormal, including some with three spindle poles. Two or three spermatid nuclei

within the same cytoplasm were attached together at their acrosomes (Varmuza et al., 1999). The observation would be consistent with the known function of PP1 in yeast and fruit flies, where it is required for sister chromatin segregation (Ohkura et al., 1989). Therefore, *PP1cγ* may be a candidate gene for male infertility *in view* of an abnormal spermiogenesis.

8. *Retinoic X receptor β (RXRβ)*

The *Retinoic X receptor β (RXRβ)* is one of three *RXRβ* s, which play critical roles in signaling pathways of retinoid, thyroid hormone, vitamin A and other yet-unidentified ligands (Chambon, 1996). *RXRβ* is expressed widely in the embryo and in adult tissues. Kastner et al. (1996) generated mouse lines in which the *RXRβ* gene was disrupted by homologous recombination. Approximately 50% of the *RXRβ* homozygous mutants died before or at birth, but those that survived appeared normal except that the male were infertile. Abnormalities were found in spermiogenesis and spermiation. It was suggested that Sertoli cells may be primarily affected in *RXRβ* null mutants, because *RXRβ* appears to be solely expressed in Sertoli cells and because Sertoli cells in *RXRβ*-deficient mice reveals histological abnormalities such as progressive accumulation of lipids. *RXRβ* may be a candidate infertile gene, especially for patients of which the Sertoli cells reveal morphological abnormalities.

9. *Calmegin*

Calmegin is a Ca^{2+}-binding protein localized on the endoplasmic reticulum membrane (Ohsako et al., 1994). In the testis, *calmegin* was expressed exclusively in germ cells of pachytene spermatocyte to spermatid stage (Masahito et al., 1997). Spermatogenesis was normal in the *calmegin*$^{-/-}$ mice, as indicated by the production of mature spermatozoa and appropriate proportion of cells in each substage. There was no difference in the size of the mutant testis or in the number of germ cells per section of a tubule counted separately according to the meiotic stage. However, homozygous mutant male mice were nearly sterile, even if copulation and vaginal plug formation were normal (Masahito et al., 1997). It turned out that sperms from *calmegin*$^{-/-}$ males were generally unable to penetrate the egg extracellular matrix. The sperms failed to adhere to the egg despite frequent collisions with zona pellucida (Masahito et al., 1997). It was proposed that *calmegin* functions as a chaperone for one or more sperm surface proteins that mediate the interactions between sperm and egg. The defective zona pellucida-adhesion phenotype of sperm from *calmegin*-deficient mice is reminiscent of certain cases of unexplained infertility in human males (Masahito et al., 1997).

10. *Fertilin*

The ADAM family is comprised of structurally related cell surface proteins proposed to have cell adhesion activity, protease activity, or both (Blobel, 1997). Fertilin is a sperm surface protein that belongs to the ADAM family. Sperm from mice lacking *fertilin β* subunit were shown to be deficient in sperm-egg membrane adhesion, sperm-egg fusion, migration from the uterus into the oviduct, and binding to the egg zona pellucida (Cho et al., 1998). These observations support direct roles of fertilin in sperm-egg plasma membrane interaction, and possibly in oviduct migration and sperm-zona binding. Therefore, *fertilin* can be a candidate gene responsible for infertile male with normal sperm counts.

11. *Angiotensin-converting enzyme (Ace)*

Angiotensin-converting enzyme (*Ace*) is a dipeptidyl carboxypeptidase that generates the vasoconstricting peptide angiotensin II and inactivates the vasolidating peptide bradykinin. It was suggested that *Ace* influences blood pressure. *Ace* has two species of transcripts: a somatic cell-specific transcript and a germ cell-specific transcript that was found only in postmeiotic spermatids. *Ace*-deficient mice appeared to be outwardly healthy with some abnormality in the kidney (Krege et al., 1995). Heterozygous males but not females had reduced blood pressure. Interestingly, all homozygous female mutants were found to be fertile, but the fertility of homozygous male mutants was greatly reduced. The finding that male mice homozygous for a disrupted *Ace* gene have impaired fertility suggests that the *Ace* gene may be responsible for male infertility

with reduced sperm numbers.

12. *Estrogen receptor (ER)*

The importance of the *estrogen receptor* (*ER*) in the function of the female reproductive system is well established, but less is known about the role of the *ER* in the male. It was known that estrogen is involved in regulating male reproductive processes. For example, prenatal or early postnatal treatment with estrogen has detrimental effects on the morphogenesis and function of the male reproductive system (Arai et al., 1983). The reproductive system appears anatomically normal in male mice homozygous for the *ER* gene (Lubahn et al., 1993). However, *ER*-deficient male mice are infertile, indicating that *ER*-mediated processes are essential for regulating male reproductive process (Eddy et al., 1996). The testis weight for *ER*-deficient mice was significantly less than that for wild-type or heterozygous mice. Serum testosterone levels were moderately higher for *ER*-deficient males than for wild-type or heterozygous males. *ER*-deficient male mice showed some tubules have a dilated lumen and a disorganized seminiferous epithelium with few spermatogenic cells or lack a lumen and contain mainly Sertoli cells. Sperm from *ER*-deficient mice have reduced motility and are ineffective at fertilizing eggs in vitro (Eddy et al., 1996). Therefore, the loss of estrogen action required for male fertility leads to reduced mating frequency, low sperm numbers, and defective sperm function.

13. *BMP8A*

Bone morphogenic proteins (BMPs) are signaling molecules of the transforming growth factor-β (TGF-β) superfamily (McPherron & Lee, 1996). They are involved in diverse regulatory mechanisms in embryonic development by controlling processes such as cell proliferation, apoptosis, specification of cell fate, and differentiation (Wall & Hogan, 1994).

BMP8A expression was detected in the decidum during pregnancy, in spermatogonia and primary spermatocytes during the initiation of spermatogenesis, and in round spermatids during the maintenance of spermatogenesis (Zhao & Hogan, 1996). All mutant males showed normal fertility initially. However, as they aged, some animals eventually became sterile. *BMP8A* is expressed at lower level than *BMP8B* during the initiation of spermatogenesis and plays a role in the maintenance of spermatogenesis (Zhao et al., 1998). Although *BMP8A* is expressed in the epidydimis, specifically in the initial segment of the caput epidydimis, the defects observed in *BMP8A* homozygous mutants are in the distal caput and the cauda regions of the epidydimis. This may suggest that either *BMP8A* protein or another growth factor produced locally in response to *BMP8A* is secreted by the initial segment of the caput and acts as a paracrine molecule on the epithelium of the distal caput and the cauda region of the epidydimis to maintain its survival (Zhao et al., 1998). It was proposed that *BMP8A* is a candidate gene with a role in maintaining spermatogenesis and the integrity of the epidydimis in the cases of human male-infertility.

14. *BMP8B*

BMP8B is expressed in male germ cells of the testis and trophoblast cells of the placenta, suggesting that it has a role in spermatogenesis and reproduction (Zhao et al., 1996). Homozygous mutant mice showed variable degrees of germ-cell deficiency and infertility. During early puberty, the germ cells of all homozygous mutants either fail to proliferate or show marked reduction in proliferation and delayed differentiation. In adults, there is a significant increase in programmed cell death of spermatocytes, leading to germ-cell depletion and sterility although Sertoli cells and Leydig cells appear relatively unaffected in mutants (Zhao et al., 1996). Therefore, *BMP8B* is required for the resumption of male germ-cell proliferation at early puberty, and for germ-cell survival and fertility in the adult. Consequently, *BMP8B* may be a candidate gene for male infertility through a disruption of cell-cell interaction between Sertoli cells and germ cells.

15. *c-Ros*

The *c-Ros* proto-oncogene encodes a tyrosine kinase receptor whose ligand has not been identified yet (Riethmacher et al., 1994). *c-Ros* is expressed specifically in a small number of epithelial cell types, including those of the epididymis. Tageted mutations of *c-Ros* in the mouse reve-

aled no visible phenotypes except male infertility (Sonnenberg-Riethmacher et al., 1996). It turned out that *c-Ros* does not affect sperm generation or function in a direct manner. The inability of *c-Ros* mutant males to reproduce is caused by a defect in regionalized differentiation of epithelial cells in epididymis during puberty (Sonnenberg-Riethmacher et al., 1996). Such defect appears to interfere with sperm maturation and the ability of sperm to fertilize *in vivo*. Interestingly, sperm isolated from *c-Ros*-deficient mice can fertilize *in vitro*. These observations suggest that *c-Ros* may be a candidate infertility gene of patients with normal sperm production. In vitro fertilization may be a solution for such cases.

16. *Desert hedgehog (Dhh)*

The hedgehog signaling pathway is essential for the development of diverse tissues during embryogenesis (Hammerschmidt et al., 1997). Signaling is activated by binding of Hedgehog protein to the multipass membrane protein, *Patched* (*Ptc* ; Tabin et al., 1997). Hedgehog consists of *Sonic hedgehog (Shh)*, *Indian hedgehog (Ihh)*, and *Desert hedgehog (Dhh)*. It is known that *Dhh* is expressed and secreted in Sertoli cells of the testis, where it is required for male germline development. *Dhh* expression is initiated in Sertoli cell precursors shortly after the activation of Sry (the sex determining gene) and persists in the testis into the adult. *Hedgehog-interacting protein (Hip)* and *Ptc-1* were expressed in the androgen-producing interstitial somatic cells (the Leydig cells) which are thought to respond to *Dhh* signaling (Bitgood et al., 1996). Female mice homozygous for a *Dhh*-null mutation show no obvious phenotype, whereas males are viable but infertile, owing to a complete absence of mature sperm. The expression of *Ptc* is also lost in *Dhh* mutants. Loss of *Ptc* expression in *Dhh* mutants suggests that *Dhh* and *Ptc-1* are thought to mediate an interaction between Sertoli and Leydig cells for normal spermatogenesis (Bitgood et al., 1996). Therefore, *Dhh* and *Ptc* have an importance on male infertility since they are required for the interaction between Sertoli and Leydig cells in spermatogenesis.

CONCLUSION

Candidate genes for male infertility are now emerging from diverse research areas, including genomic studies of the Y chromosome and mouse genetics studies. It is apparent that genetic defects that cause male infertility originate from various genes located both on the Y chromosome and autosomes. Therefore, it is currently important to collect a full repertoire of infertility genes from the human genome.

The next step may be to develop diagnostic methods for genetic defects of male infertility. It is likely that a great deal of idiopathic infertility stems from genetic causes. It would be critical to diagnose a specific gene that is responsible for each infertility patient for decision on the strategy of assisted reproduction. Moreover, the progeny can be aware of possible problems in their ability of reproduction in the future. Recent progress in the microarray technique will provide useful tools for identification of infertility genes and for further diagnosis of genetic causes.

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