Human Reproduction Update, Vol.8, No.2 pp. 183–198, 2002

# Selected genetic factors associated with male infertility

**Trieu Huynh, Richard Mollard and Alan Trounson[1]**

Monash Institute of Reproduction and Development, Centre for Early Human Development, 27–31 Wright Street, Clayton, Victoria 3168, Australia

[1]To whom correspondence should be addressed. E-mail: mary.lo@med.monash.edu.au

**Studies into the mechanisms underlying spermatogenesis, the process by which spermatogonia undergo meiosis to become spermatozoa, have identified a number of genetic determinants of male infertility. Indeed, a more comprehensive knowledge of the genetic regulation of spermatogenesis has alleviated the dependence on the use of idiopathic infertility as a classification for sterile men for whom a cause for their infertility is unknown, as genetic factors become more accountable for this phenotype. This review focuses on selected areas implicated in male infertility including: (i) autosomal and sex chromosomal abnormalities; (ii) genetic disorders associated with impaired gonadotrophin secretion or action; (iii) microdeletions within regions of the Y-chromosome containing candidate gene families for spermatogenesis; (iv) the genetic nexus between cystic fibrosis and congenital bilateral absence of the vas deferens; and (v) insights into human infertility as gleaned from animal studies into mechanisms involving the *Bcl-2* family of apoptosis regulators and the interaction between the *c-kit* encoded tyrosine kinase receptor and its ligand, stem cell factor. As significant advances continue to further knowledge of the genetic basis of male infertility, such as those leading to an understanding of the aforementioned areas, greater progress can be made to rectify or at least ameliorate social stigmas associated with sterility.**

*Key words:* chromosomal abnormalities/cystic fibrosis and congenital absence of the vas deferens/male infertility/spermatogenic function/Y microdeletions

## TABLE OF CONTENTS



## Introduction

Spermatogenesis depends firstly upon the organized development of the testes, a process that involves several phases beginning at embryonic day ~11.5 in the mouse or embryonic week 4–6 in the human (Meehan *et al.*, 2001). At this stage, primordial germ cells (PGCs), located within the primitive genital ridge, are induced to proliferate by Sertoli cells (Wang *et al.*, 1998). Other influences including paracrine, endocrine and intracellular signals determine the fate of the PGC, with the majority undergoing apoptosis. The remaining PGCs differentiate into gonocytes that remain dormant until the initiation of the first wave of spermatogenesis (Print and Loveland, 2000).

In humans, the first wave of spermatogenesis occurs between birth and the first 6 months after birth. This stage is initially characterized by the mitotically-arrested gonocytes re-entering the cell cycle and differentiating into spermatogonia that subsequently undergo proliferation (Kaufman, 1992; Meehan *et al.*, 2001). Mice experience this event during the first 5 days post partum (pp). Whilst some of the spermatogonia form a self-regenerating population of spermatogonial stem cells, the majority become spermatocytes that are subsequently directed to undergo meiosis. This takes place at ~10 days pp in mice and at puberty in humans. Haploid spermatids appear after 20 days pp in murine spermatogenesis with spermatozoa becoming evident in the lumen of seminiferous tubules by day 35 pp (McCarrey, 1993). In humans, the onset of puberty brings with it an increase in gonadotrophin and androgen levels that signals the initiation of spermatocyte development and the emergence of haploid spermatids (Print and Loveland, 2000). Adult mice and humans experience continuous waves of spermatogenesis following this initial upsurge of spermatozoa, with spermatogonial stem cells serving to repopulate the germ cell pool.

The first wave of spermatogenesis is also associated with the proliferation and maturation of the somatic cells of the testis. Murine somatic Sertoli cells, for example, proliferate up until day 16 pp (Orth, 1982) and provide nutrition, adhesion and transport functions to the adjacent germ cells in addition to fate-determining signals (Russell *et al.*, 1990) such as stem cell factor (SCF) (see section: Stem cell factor and c-kit tyrosine kinase receptor interactions in the mammalian testis). Other somatic cells intimately involved in spermatogenesis are the Leydig cells which are the source of androgens, the class of male sex steroids vital to development and spermatogenesis. Leydig cells can be dichotomized into fetal and adult varieties, with the former present at birth and maintained as a stable population throughout life. The latter emerge from a separate pool of precursor cells and are first identifiable at day 10 (Mendis-Handagama *et al.*, 1987). By day 25, the contractile peritubular myoid cells enveloping the seminiferous tubules assume a flattened phenotype and exude the basal lamina components typical of the mature testis (Palombi *et al.*, 1992).

The success of this biological process of sperm production depends on a meticulously regulated cascade of developmental genes that co-ordinate spermatogonial cell proliferation, chromosomal reduction divisions that produce haploid daughter cells and the morphological differentiation of these daughter cells into mature sperm (Hargreave, 2000). It has been estimated that >2000 genes are involved in the control of masculine development and reproduction (Hargreave, 2000), and it has been further postulated that mutations in any of the genes in the various associated conduits may have devastating effects on testis development, normally manifesting as infertility. Thus, the difficulty in establishing definitive genetic causes of male infertility cannot be understated.

Notwithstanding this complexity, significant progress has been made with defects in male reproduction having been associated with a variety of genetic factors. It is the intention of this review to dissect several of these areas, concentrating on genetic abnormalities currently accepted as being involved in human male infertility as well as mechanisms speculated to be involved, as gleaned from animal models. To begin with, autosomal and sex chromosomal abnormalities, in particular Klinefelter's syndrome, will be considered as a high proportion of infertile men possess such genetic anomalies, more so than in the general population. Furthermore, disorders affecting gonadotrophin secretion and action have also been demonstrated to adversely affect male fertility, manifesting as a failure to reproduce. Thus conditions involving inactivating mutations of LH and FSH genes as well as Kallman's and Prader–Willi syndromes will also be discussed. Attention will similarly be paid to microdeletions within regions of the Y-chromosome that have been shown to contain candidate gene families for spermatogenesis such as *USP9Y*, *RBMY*, and *DAZ*, corresponding to the *AZFa*, *AZFb* and *AZFc* regions of Yq respectively. Absence of such segments has been identified in a number of men displaying phenotypic normality save for their infertility, suggesting that these deletions may be a genetic basis responsible for male sterility. Striking phenotypic similarities between cystic fibrosis (CF) and congenital bilateral absence of the vas deferens (CBAVD), a condition frequently observed in cases of obstructive azoospermia, have prompted suggestions of a shared genetic origin. As CF is the most predominant genetic disease amongst Caucasians and affects a relatively large proportion of the population, a detailed discussion of the association between CF and male infertility arising from CBAVD is justified. Insights into human male infertility have also been furthered through research using animal models. For example, disequilibrium in the expression of apoptosis regulators, the *Bcl-2* family, has also been observed to induce male infertility in mice and thus warrants discussion because of possible similarities in human spermatogenesis. Similarly, the interaction between the *c-kit* tyrosine kinase receptor, and its ligand, SCF, has been implicated in PGC migration, spermatogonial adhesion and proliferation, and the inhibition of apoptosis of germ cells in the testis.

### *Definitions of infertility*

It is generally accepted that ~90% of fertile couples successfully conceive within 1 year (Tietze, 1956, 1968). From this observation, couple infertility has been commonly defined as the inability to conceive after 12 months of regular intercourse in the absence of contraceptives (Krausz and Forti, 2000), and based on this classification, the prevalence of infertile couples in Western countries has been determined to be 10–15% (World Health Organization, 1987).

In a multi-centre study conducted by the World Health Organization into the diagnosis and management of male infertility (World Health Organization, 1987), it was concluded that in 20% of cases of couple infertility, the problem could be attributed predominantly to the male (pure male factor). Pure female factor, in which infertility was associated principally with the female partner, was found in 38% of cases, whilst in 27% both partners presented abnormalities manifesting as infertility. Of the remaining 15% of infertile couples, no definitive cause was identified.

### *Common semen anomalies detected in infertile men*

Male factor infertility has been linked with a multitude of irregularities including sperm number, motility and morphology. Table I defines the normal values of semen parameters whilst Table II represents semen anomalies regularly identified in infertile men. In assessing semen quality, fertility clinics are also beginning to focus on sperm DNA integrity in addition to these characteristics, with the development of a rapid, reliable and practical test for sperm chromatin structure having recently been made available. Damaged DNA in the individual sperm that fertilizes an oocyte can result in adverse affects on fetal development and health throughout adult life. Therefore the availability of the sperm chromatin structure assay (SCSA), which utilizes the metachromatic features of Acridine Orange, a DNA probe and flow cytometry (Evenson and Jost, 2000), is useful in ascertaining sperm nuclear chromatin integrity and is thus beneficial as an indicator of sub/infertility. Moreover, SCSA provides clinicians with a means of rapidly ascertaining an idea of the suitability of sperm for assisted reproductive techniques.

The first four semen anomalies defined in Table II, i.e. azoospermia, oligozoospermia, asthenozoospermia and teratozoospermia, are present in approximately half the cases of couple infertility and in almost 90% of infertile males. In ~10% of cases, normal semen parameters are exhibited yet infertility still results. In such circumstances, it has been postulated that specific

**Table I.** Normal values of semen parameters (World Health Organization, 1992)

| Parameter | Value |
|---|---|
| Volume | >2 ml |
| pH 7.2–8.0 | |
| Sperm concentration | $>20\times10^6$ spermatozoa per ml |
| Total sperm count | $>40\times10^6$ spermatozoa per ejaculate |
| Motility | >50% with forward progression or >25% with rapid progression at 60 min after ejaculation |
| Morphology | >30% with normal forms |

**Table II.** Nomenclature for pathological findings in semen analysis (World Health Organization, 1992)

| Semen anomaly | Description |
|---|---|
| Azoospermia | No spermatozoa in the ejaculate |
| Oligozoospermia | Sperm concentration $<20\times10^6$ per ml |
| Asthenozoospermia | <50% of sperm exhibit normal forward motility or <25% demonstrate any motility |
| Teratozoospermia | <30% with normal forms |
| Oligoasthenoteratozoospermia | Disturbance in all three variables |
| Aspermia | No ejaculate |

metabolic (Huszar *et al.*, 1992) and membrane defects (Calvo *et al.*, 1989; Mendoza, 1992) of spermatozoids leads to this inability to fertilize.

In >70% of cases, a definitive cause of male infertility can usually be ascertained (Krausz and Forti, 2000); however, no cause for infertility can be found in the remaining 30%, rendering such cases idiopathic. Notwithstanding this assessment, no comprehensive definition for idiopathic infertility exists for a variety of reasons, including possible variation in the intensity of diagnosis programmes between fertility clinics and the considerable variability of spermatogenic impairment between patients, which often makes it difficult to establish a direct cause and effect relationship. Therefore, though a considerable number of infertile men go without definitive answers as to the cause of their inability to reproduce, it is becoming increasingly apparent that in the majority of cases of idiopathic infertility, an underlying genetic abnormality may provide an explanation.

## Chromosomal abnormalities and male infertility

The possible association between chromosomal abnormalities and male infertility became evident following the results of the first large karyotype survey involving subfertile males (Kjessler, 1974; Chandley, 1979). In this screen of 6982 individuals it was observed that in comparison with the general population, infertile men exhibited a higher prevalence of chromosomal anomalies (5.3 compared with 0.6%). More specifically, the incidences of sex chromosomal and autosomal abnormalities in infertile men were observed to be 15- and 6-fold greater respectively than those found in the general population (Bhasin *et al.*, 2000). Similar studies have also suggested that of the ~5% of infertile males carrying chromosomal abnormalities, 4% involve the sex chromosomes with the remaining 1% associated with autosomal irregularities (Bhasin *et al.*, 2000).

Klinefelter's syndrome, the most common chromosomal disorder associated with male infertility, has been reported to occur in ~1 in 500 newborn males (Bielanska *et al.*, 2000). In ~93% of cases, the syndrome is characterized by a 47,XXY chromosome complement; however, variants such as 47,XXY/46,XY mosaicism; 48,XXXY; 48,XXYY; and 49,XXXXY have also been reported to manifest Klinefelter's symptoms (Bielanska *et al.*, 2000). With respect to infertility, the 47,XXY karyotype has a high incidence amongst sterile men, with 11% of azoospermic and 0.7% of oligozoospermic men possessing this chromosome complement.

The classical symptoms of this disorder include extremely small (<5 ml), firm testes, with eunuchoid habitus, gynaecomastia, elevated levels of FSH and azoospermia (Bhasin *et al.*, 2000). Whilst this last symptom is considered to be a rule amongst Klinefelter patients of a 47,XXY orientation, oligozoospermia has been identified in men with mosaicism who have severely reduced, though nonetheless detectable, levels of germ cells in their testes. Furthermore, rare cases of fertility and proven paternity have also been reported amongst oligozoospermic Klinefelter patients (Bhasin *et al.*, 2000; Krausz and Forti, 2000).

Testicular histology in men with the most common variety of Klinefelter's syndrome (i.e. 47,XXY) reveals a hyalinization of the seminiferous tubules and the absence of spermatogenesis. Patients with mosaicism are less affected, with the majority having normal size testes, experiencing spermatogenesis at puberty. However, their opportunity to reproduce is temporary with progressive deterioration and hyalinization of the seminiferous tubules occurring shortly after puberty and resulting in eventual infertility. This tubular dysgenesis appears inconsistent in some men, with eroded tubules being surrounded by apparently normal tubules in some cases. The function of Leydig cells is also impaired as a result of Klinefelter's syndrome, though increased levels of these cells are present in the testes (Bhasin *et al.*, 2000).

Different opinions within the literature exists with respect to whether the germ cells from a 47,XXY male can proceed through mitosis and meiosis to generate XX and XY hyperhaploid gametes. The general consensus has been that the activity of more than one X chromosome is detrimental to the survival of the male germ cell. This notion, however, has recently come under scrutiny through studies involving fluorescent in-situ hybridization (FISH) of spermatozoids from mosaic 47,XXY/46 XY patients (Cozzi *et al.*, 1994; Chevret *et al.*, 1996; Martini *et al.*, 1996). An increased frequency of hyperhaploid 24,XY from such patients warrants the consideration that such cells may indeed possess a meiotic capacity. Nevertheless, it is still debatable whether the hyperhaploid spermatozoa arose through meiotic division of XXY germ cells or from an elevated rate of meiotic non-disjunction (Bhasin *et al.*, 2000).

Structural abnormalities that have been frequently observed in infertile men include reciprocal translocations, Robertsonian translocations, paracentric inversions and marker chromosomes. Heterozygotes for autosomal translocations have been found to be seven times more prevalent in infertile male populations compared with newborns (Krausz and Forti, 2000). Similarly, carriers of marker chromosomes are eight times more frequent

amongst infertile males (De Braekeleer and Dao, 1991). In the majority of cases of Robertsonian translocations (60%), a (13:14)-translocation is found. This anomaly is frequently detected in oligozoospermic males, but is a rare occurrence in azoospermic patients. Though it has been established that there is a marked increase of the frequency of this type of translocation among infertile males compared with newborns, the exact contribution of this type of chromosomal defect remains unclear. Chandley, for example, identified Robertsonian translocations in normospermic fertile males in the same pedigree (Chandley, 1975). Infertile male populations also experience chromosomal inversions more frequently, with this type of abnormality having been found to occur 13 times more often in these men than in newborns. Furthermore, it has been suggested that paracentric inversions in chromosomes 1, 3, 5, 6 and 10 might interfere with meiosis, resulting in a decreased capacity to produce sperm and thus infertility (Krausz and Forti, 2000).

## Genetic disorders involving impaired gonadotrophin activity associated with male infertility

Genetic disorders affecting the secretion and action of gonadotrophins have also been reported to give rise to male infertility. These generally fall into three broad categories: (i) disorders involving hypothalamic GnRH secretion or action; (ii) primary disorders of pituitary LH and FSH secretion and action; and (iii) pituitary development disorders.

As LH and FSH are trophic hormones for the testes and ovaries, reduced secretion of these gonadotrophins (hypogonadotrophism) results in inadequate functioning of the sex organs (hypogonadism) (Bhasin *et al.*, 2000). Patients with hypogonadotrophic hypogonadism may exhibit either sex steroid (androgen in the male and estrogen in the female) deficiency or infertility due to disrupted germ cell development or both.

The severity of symptoms associated with androgen deficiency depends on both the time of onset as well as the magnitude of gonadotrophin deficiency. Inadequate androgen secretion during gestation may prompt a failure of Wolffian structure development, ambiguity of the external genitalia, hypospadias, microphallus or a mixture of these symptoms (Bhasin *et al.*, 2000). In cases of isolated hypogonadotrophism, the fetal testis is stimulated by placental HCG to produce adequate levels of androgen during the early stages of fetal development. Thus, most patients with congenital GnRH deficiency present normal Wolffian structures and external genitalia. During the latter stages of pregnancy, however, the fetal gonad comes under the control of fetal pituitary LH and FSH and, because testicular descent is partially dependent on androgen activity, acute deficiency of these hormones during this phase may result in undescended testes and microphallus.

Androgen deficiency between birth and puberty normally results in delayed or arrested sexual development, with such individuals experiencing delayed adolescence. Furthermore, men afflicted by pre-pubertal androgen deficiency retain their high-pitched voice and do not develop the male pattern temporal recession of the hairline.

Post-puberty androgen deficiency is exemplified by a deterioration of the secondary sex characteristics, libido impairment, sexual dysfunction, loss of muscle mass, increased fat mass and infertility, with such changes presenting themselves surreptitiously over a protracted period of time.

Insensitivity of the androgen receptor (AR) has also been linked with impaired sperm production and thus male infertility (Tut *et al.*, 1997; Dowsing *et al.*, 1999). The AR gene contains two polymorphic trinucleotide repeat loci: $[CAG]_n$ and $[GGC]_n$. These code for a polyglutamine and a polyglycine tract respectively and are located within the transactivation domain of the AR protein (Lubahn *et al.*, 1989). In males, the excessive expansion of the polyglutamine region leads to spinal bulbar muscular atrophy, a fatal neuromuscular disorder presenting impaired development of the secondary sex characteristics also oligozoospermia or azoospermia, testicular atrophy and reduced fertility (Arbizu *et al.*, 1983). It is generally accepted that in individuals where there are $\geqslant$40 CAG repeats, this condition is manifested resulting from a decreased functional competence of AR as glutamine tracts extend (Dowsing *et al.* 1999). However, Tut and colleagues demonstrated that lesser expansions of such repeats manifest as ameliorated forms of the disease, leading to infertility without muscular dysfunction (Tut *et al.*, 1997).

### *Hypothalamic disorders involving disrupted GnRH secretion or action*

The first to describe a syndrome defined by postponed or arrested sexual development and anosmia were Kallman *et al.* who identified these symptoms to be a result of selective gonadotrophin deficiency arising from a defect in GnRH secretion (Kallman *et al.*, 1944). The principal defect was concluded to be hypothalamic with the impaired gonadotrophin secretion being auxiliary to the hypothalamic aberration in GnRH secretion. Other common symptoms associated with idiopathic hypogonadotrophin hypogonadism (IHH) include colour blindness, cleft lip and palate, cranial nerve defects, cryptorchidism and optic atrophy.

Significant diversity in the clinical presentation of IHH has been reported with the phenotype, to a large extent, dependent on the degree of GnRH depletion. The most severe deficiency may manifest as a complete absence of pubertal development, sexual infantilism and varying levels of hypospadias and undescended testes (Crowley *et al.*, 1985; Spratt *et al.*, 1987; Waldstreicher *et al.*, 1996). GnRH deficiency of a lower magnitude may result in varying degrees of impairment of sexual development, proportional to the deficiency of gonadotrophin.

Genetically, IHH is a heterogeneous disorder with only one-third of IHH individuals possessing a positive family history. Among this group, ~20% have an X-linked pattern of inheritance, one-third acquire the disorder in an autosomal recessive fashion, while the remainder inherit the condition in an autosomal dominant manner (Spratt *et al.*, 1987; Waldstreicher *et al.*, 1996). Through physical and genetic mapping, the X-linked form of Kallman's syndrome has been localized to a mutation in the *KALIG-1* (Kallman's syndrome interval-1) gene on Xp22.3 (Ballabio *et al.*, 1989; Hardelin *et al.*, 1993). It has been suggested that the protein encoded by this gene regulates the migration of the GnRH and olfactory neurons and their development. Though deletions of the *KALIG-1* gene have only been reported in a limited group of men with IHH, point mutations, most of which are located in the fibronectin-III domain of the *KALIG-1* gene, have been described (Georgopoulos *et al.*, 1997; Maya-Nunez *et al.*, 1998). Such mutations in the *KALIG-1*

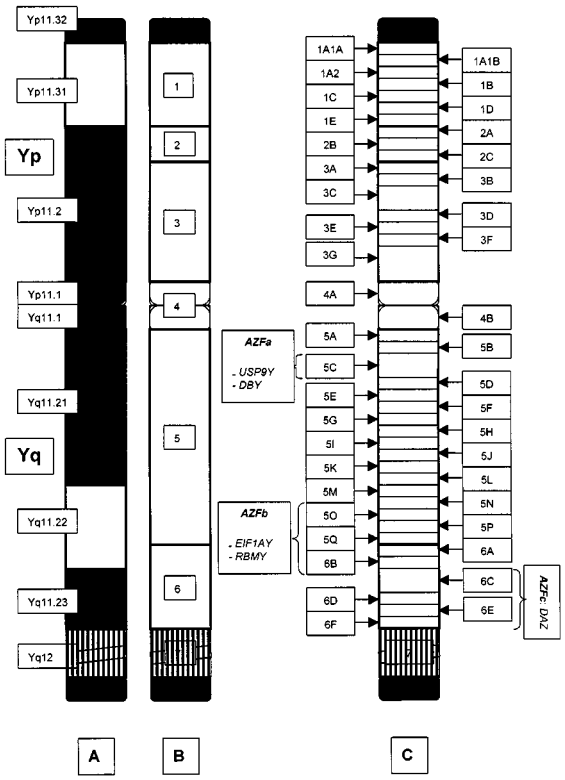


**Figure 1.** (**A**) Schematic representation of the cytological bands of the Y chromosome. (**B**) Vergnaud map of the Y chromosome showing the seven deletion intervals [reproduced with permission from Vergnaud *et al.* (1986). *Am. J. Hum. Genet.* **38**; 109–124, published by The University of Chicago Press, Chicago, IL, USA]. (**C**) Vollrath map dividing the Y chromosome into 43 subintervals (adapted from Vollrath et al., 1992). The three AZF regions and the candidate genes of each are also included.

gene are believed to be responsible for only a small number of patients with the X-linked form of IHH, and it is further proposed that additional, as yet undetermined, X-linked genes are also involved in other forms of Kallman's syndrome.

Prader–Willi syndrome is another example of a hypothalamic disorder associated with impaired GnRH activity. Symptoms of this condition generally include obesity, hypotonic musculature, mental retardation, hypogonadism, short stature, and small hands and feet, though hypogonadism, cryptorchidism and micropenis are also common (Cassidy and Schwartz, 1998). From a histological perspective, the testis is immature and devoid of germ cells, although Sertoli cells and diminutive tubules are nonetheless present. Individuals with Prader–Willi syndrome also demonstrate varying degrees of gonadotrophin deficiency leading to variation in phenotype. Prader–Willi syndrome is a disorder of genomic imprinting that is frequently caused by deletions of the proximal portion of paternally derived chromosome 15q (Cassidy and Schwartz, 1998). Because the maternally derived copies responsible for the condition remain silent, the deletion of the paternally derived copy of the normally active genes generates the disease.

### *Defects in pituitary LH and FSH receptor genes*

Infertility has also been associated with inactivating mutations of LH and FSH receptor genes whereby individuals experiencing such conditions present hypogonadism and Leydig cell hypoplasia (Bhasin *et al.*, 2000). Resistance to LH action alone, arising as a result of inactivating mutations of the LH receptor, has also been reported in a number of studies. Men with LH receptor mutations exhibit a variety of phenotypic abnormalities including feminization of the external genitalia in 46,XY males, Leydig cell hypoplasia, primary hypogonadism and delayed sexual development. In one case of Leydig cell hypoplasia and hypogonadism, a T to A mutation was identified in position 1874 of the LH receptor gene. Testicular histology in this individual exposed the absence of mature Leydig cells in the interstitium with the seminiferous tubules developing an excessively viscous basal lamina and spermatogenic arrest at the elongated spermatid stage. Inactivating mutations of the FSH receptor gene have also been described with varying degrees of hindrance to spermatogenesis and fertility observed amongst this group.

## Microdeletions of the Y chromosome and implications for male infertility

### *Y chromosome structure and gene content*

The human Y chromosome consists of a short and a long arm, denoted Yp and Yq respectively (refer to Figure 1). Located at the distal portions of these arms are areas of sequence identity to the X chromosome that permit pairing and recombination during male meiosis, and are thus referred to as the pseudoautosomal regions (PARs). The region beyond the PARs that escapes recombination is called the non-recombining region of the Y chromosome and comprises several repetitive sequences that are presumed to be either homologues to regions on the X chromosome or Y-specific (Foresta *et al.*, 2001). Both Yp and the proximal portion of Yq consist of euchromatin, whilst the distal segment of Yq is composed of heterochromatin. This latter region generally varies in length and normally comprises between one-half to two-thirds of Yq. Thus, the long arm of the Y chromosome is cytogenetically dichotomized into a euchromatic proximal region (Yq11, segregated further into Yq11.1, 11.21, 11.22 and 11.23) and a heterochromatic distal region (Yq12). The euchromatic short arm is denoted Yp11.

Initial attempts at constructing a linkage map of the human Y chromosome were hindered by the absence of meiotic recombination within most regions of the chromosome. Consequently, mapping has instead been based on naturally occurring deletions resulting in an interval map separating the Y chromosome into seven segments (Vergnaud *et al.*, 1986), with the short arm and the centromeric region containing the first four intervals. The euchromatic portion of the long arm is represented by intervals 5 and 6, distal to proximal, while the heterochromatic region of distal Yq is considered interval 7. Vollrath *et al.* further delineated the seven-interval map into 43 subintervals, resulting in the most commonly used map of the human Y chromosome (Vollrath *et al.*, 1992).

Evidence of a nexus between spermatogenic failure and an underlying genetic cause was presented by Tiepolo and Zuffardi who reported microscopically detectable deletions in the distal portion of Yq in six azoospermic men taken from a screen of 1170 cases (Tiepolo and Zuffardi, 1976). In all these patients, absence of spermatozoids in the ejaculate was the only symptom observed, suggesting that factors influencing human spermatogenesis were

located in this distal region (Yq11.23) which later came to be referred to as 'azoospermia factor' (*AZF*).

Vogt and colleagues in a study involving 19 infertile males with non-obstructive severe oligozoospermia or azoospermia, identified two men possessing microdeletions in distal Yq that were apparently non-overlapping (Vogt *et al*., 1992). A subsequent investigation by Ma *et al.* described similar non-overlapping microdeletions in the interval 6 region in this same area (Ma *et al.*, 1993). Cumulatively, these findings suggested the existence of a multitude of loci located in this domain that may be associated with infertility.

Evidence to confirm the accuracy of this preliminary conclusion was published by Vogt and co-workers who, after screening 370 men manifesting idiopathic oligozoospermia or azoospermia for deletions of 76 DNA loci in Yq11, identified 12 individuals presenting de-novo microdeletions and one patient harbouring an inherited deletion (Vogt *et al.*, 1996). It was concluded that these deletions corresponded to different sub-regions within Yq11.

These findings lead to the characterization of three separate non-overlapping regions required for spermatogenesis that came to be referred to as *AZFa*, *AZFb* and *AZFc*. The first of these azoospermia factors, *AZFa*, is located in the proximal portion of deletion interval 5 (subinterval 5C) (refer Figure 1C). *AZFb* extends from distal deletion interval 5 to the proximal end of deletion interval 6 (subinterval 5O–6B). Estimation as to the size of these two regions fall between 1 and 3 Mb with a definitive assessment not forthcoming due to blocks of highly repetitive sequences consistently leading to underestimation (McElreavey *et al.*, 2000). *AZFc* lies immediately proximal to the heterochromatin region of the Y chromosome, between subintervals 6C and 6E, and is ~1.4 Mb in size, though a concise characterization of this region is again hindered by sequence repetition. A fourth *AZF* subregion, positioned between *AZFb* and *AZFc*, was also recently proposed and named *AZFd* (Kent-First *et al.*, 1999).

Studies into Y chromosome deletions have also demonstrated that whilst genes within the non-combining region play a critical role in male fertility, erasure of such loci are not exclusively associated with azoospermia (Reijo *et al.*, 1996). Indeed, microdeletions within the AZF regions have been linked to a variety of testis histological profiles including Sertoli-cell-only syndrome, spermatogenic arrest and morphological abnormalities of post-meiotic germ cells. Vogt and colleagues suggest these symptoms arise as a result of deletions of the *AZFa*, *AZFb* and *AZFc* regions respectively (Vogt *et al*., 1996).

### *AZFa candidate genes associated with spermatogenesis*

The first gene to be identified within the *AZFa* region and subsequently demonstrated to be absent in infertile patients was *DFFRY* (Drosophila fat facets related Y), named as a consequence of homology to the Drosophila developmental gene fat facets (*faf*) (Foresta *et al.*, 2001). Recently renamed *USP9Y* (ubiquitin-specific protease 9, Y chromosome), it is a single copy gene that appears to function as a C-terminal ubiquitin hydrolase and rather than having specific expression within the testes, *USP9Y* is ubiquitously expressed in a wide variety of tissues (Foresta *et al.*, 2001). In humans, *USP9Y* is mapped to Yq11.2 and Xp11.4 (Jones *et al.*, 1996; Brown *et al.*, 1998) and also has an X-homologue that avoids X-inactivation.

Though *USP9Y* constitutes less than half of *AZFa*, the majority of infertile males who exhibit deletions within this section are generally devoid of the entire interval that comprises the azoospermia factor. On the basis of such findings, it is proposed that other gene(s) within this region are accountable, either individually or in concert with *USP9Y*, for the spermatogenic anomalies experienced by *AZFa*-deleted individuals. Furthermore, comparative mapping studies have demonstrated that two other XY homologous genes are also located within *AZFa*, thus suggesting a possible involvement in spermatogenesis. These two candidate genes are *DBY* (dead box on the Y) (Lahn and Page, 1997) and *UTY* (ubiquitous TPR motif on the Y) (Mazeyrat *et al.*, 1998), though more recently, a novel expressed sequence denoted *AZFaT1* has also been localized to this area (Sargent *et al.*, 1999). These genes appear to be ubiquitously expressed, unlike their mouse homologues such as *Dffry* which is testis specific (Brown *et al.*, 1998).

Preliminary studies involving patients with deletions within the *AZFa* region suggested that deficiency of *USP9Y* or *AZFaT1*, or both, manifested as male infertility, with the additional absence of *DBY* believed to exacerbate the condition (Sargent *et al.*, 1999). Convincing evidence of *USP9Y* as a spermatogenesis gene has also recently emerged with the discovery of a truncated protein in an azoospermic man arising from a 4 bp deletion (Sun *et al.*, 1999). Nevertheless, as a result of extensive deletion and expression analysis of deletion intervals 5C and 5D in a selected panel of infertile patients, a more refined map of *AZFa* has been assembled which has consequently alluded to *DBY* representing the major spermatogenesis gene of this region (Foresta *et al.*, 2000). *DBY* is more frequently deleted than *USP9Y* and, in addition to ubiquitous expression, exhibits transcripts unique to the testis. Additional support that DBY protein participates in human spermatogenesis lies in its significant homology to the mouse protein PL10 which is testis-specific and expressed only in germ cells (Foresta *et al.*, 2000). Though it is known that *DBY* comprises 17 exons and encodes for a putative ATP-dependent RNA helicase, its exact function in male germ cell development is as yet undetermined.

### *Candidate genes of the AZFb region*

At present, two genes have been mapped to *AZFb*, a region that corresponds to deletion subintervals 5O–6B. The first, *EIF1AY* (translation initiation factor 1A, Y isoform), encodes a Y isoform of eIF-1A, a ubiquitously expressed translation initiation factor that has an X chromosome homologue (Lahn and Page, 1997). Though the role of *EIF1AY* in spermatogenesis is at yet unknown, the fact that no deletion specifically eliminating this gene has been discovered has lead to the general consensus that this is not an *AZFb*-candidate gene. Nevertheless, because *EIF1AY* possesses abundant testis-specific transcripts, in addition to ubiquitous transcripts (Lahn and Page, 1997), such a conclusion cannot be confirmed as it appears that it may indeed have a role in the *AZFb* phenotype that has not yet been determined.

Also located within the *AZFb* region is *RBMY* (RNA-binding motif on Y), a multicopy gene family thought to consist of 30–40 members, some of which are pseudogenes (Ma *et al.*, 1993; Prosser *et al.*, 1996). Formerly referred to as *YRRM* (Y-specific RNA recognition motif), numerous *RBMY* genes have been detected across both arms of the Y chromosome; however, only

genes within *AZFb*, specifically deletion interval 6B, produce detectable levels of the protein.

*RBMY* genes and pseudogenes can be further divided into several subfamilies (*RBMY1* to *RBMY6*). The predominant *RBMY1* subfamily comprises at least seven members that differ in sequence homology by between one and seven bases, and are arrayed in tandem in the *AZFb* region, in proximal deletion interval 6 (Prosser *et al.*, 1996; Chai *et al.*, 1997, 1998). These genes are expressed exclusively in germs cells and encode nuclear proteins that contain a highly conserved 90 amino acid RNA-binding motif (RBM) as well as four copies of a 37 residue peptide internal tandem repeat structure termed the 'SRGY box' due to its high content of Ser-Arg-Gly-Tyr amino acids. *RBMY2* genes share 88% homology with their *RBMY1* relatives, and carry the RBM and a single copy of the SRGY box.

Soulard and colleagues revealed RBMY1 protein sequences to present 67% peptide-similarity to the autosomally expressed HNRNPG, a wide-ranging nuclear glycoprotein with RNA binding activities mapped to human chromosome 6p12, but with as yet no known biological function (Soulard *et al*., 1993). Furthermore, Delbridge and coworkers identified an additional homologue to *RBMY* on human Xp26 which was aptly named *RBMX* to reflect its location and homology with the *RBMY* gene family (Delbridge *et al*., 1999). Like other gene compliments on the X and Y chromosomes, it seems *RBMX* retained a widespread function whilst *RBMY* adopted a male-specific function in spermatogenesis (Delbridge *et al.*, 1999). The fact that the HNRNPG protein, as well as RBMY homologues identified in other mammals including mice and marsupials, contain only one SRGY box led to the proposal that the *RBMY* gene family may have emerged from a transposition of an *HNRNP G*-like ancestral gene to the Y chromosome, followed by a succession of internal amplifications of one of the exons, and then multiplication of the entire gene (Delbridge *et al.*, 1997,1998).

Expression of the human RBMY and murine Rbm proteins is unique to germ cells in the testis. *RBMY1* has been observed to co-localize with a number of known splicing factors at certain stages of spermatogenesis. Thus, it has been proposed that *RBMY1* participates in mRNA processing including the moderation of mRNA splicing (Elliott *et al.*, 1998). The deletion of some members of the *RBMY* gene family has also been detected in infertile men exhibiting severe oligozoospermia or azoospermia. The loss of *RBMY* members in the *AZFb* region appears directly associated with spermatogenic arrest at meiosis.

It is still contentious as to whether only one copy of the *RBMY* gene is sufficient for the completion of normal spermatogenesis in humans. Studies involving marsupials have demonstrated that in these animals, normal spermatogenesis can be effected with only one copy of *RBMY* (Delbridge *et al.*, 1997). Further and alternatively, multiple copies of genes may be indispensable where large amounts of products are required for the manufacture of large amounts of sperm. Structural, functional and evolutionary arguments exist maintaining that repeated genes can be beneficial either through dosage repetition or variant repetition, or both. Dosage repeated genes are characterized by high copy numbers of identical sequences in tandem repeated clusters and it has been suggested that such replication facilitates an organism's need for a large amount of product with examples including ribosomomal genes, transfer RNA genes and histone genes (Kedes, 1979). Variant repeat genes such as the globin genes, actin genes and immunoglobulin genes do not share exact homology but are highly related and might have arisen from the duplication and divergence of a common ancestral gene (Long and Dawid, 1980).

Recent studies involving yeast artificial chromosome (YAC) contigs have provided evidence to suggest that the human *RBMY* family arose from both variant repetition and dosage repetition (Prosser *et al.*, 1996). Humans and other primates have been shown to possess more copies of *RBMY* genes than other mammals, a possible cause for their reduced capacity to produce sperm when compared with other mammals such as rats and mice. Thus, more copies of such genes are required in order to compensate for this inefficiency. The deletion of certain gene copies (dosage effect) may therefore manifest as oligozoospermia rather than azoospermia due to insufficient levels of many transcripts as opposed to the complete absence of any particular one (Karsch-Mizrachi and Haynes, 1993).

An inevitable consequence of having a high number of genes is a corresponding elevated rate of mutation. This is perhaps a reason for the variable phenotypes (variable sperm counts) observed amongst oligozoospermic individuals. Such dosage effect has also been reported in other Y-chromosome genes such as the multicopy gene *Y353/B*, a candidate regulator of spermiogenesis in mice (Conway *et al.*, 1994). Its putative involvement in sperm morphology maturation derived from the observed increased incidence of abnormal sperm heads in mice with reduced *Y3535/B* copy number.

In order to obtain a greater comprehension of the role of the *RBMY* gene family, a comparative detailed study of the gene organization in other species is essential. The deletion of most copies of the *Rbmy* gene in mice, for example, reveals heightened levels of abnormal sperm development (Mahadevaiah *et al.*, 1998). Studies such as these should provide further insights into the function of *RBMY* in humans.

### *Candidate genes of AZFc*

On the basis of Southern blot analysis, it was originally considered that *DAZ* was a single-copy gene (Reijo *et al.*, 1995), however, subsequent high resolution FISH analysis lead to the revised conclusion that there are between three and seven copies of the gene clustered in the *AZFc* region of the Y chromosome. *DAZ*, like the *RBM* family, encodes a testis-specific protein containing a single RNA-binding motif and between eight and 24 copies of a 24 amino acid sequence named the 'DAZ repeat' (Reijo *et al.*, 1995; Yen *et al.*, 1997).

Human Y-linked *DAZ* is homologous to an autosomal gene located at chromosome 3p24 that contains a single *DAZ* repeat and is thus named *DAZL1* (DAZ like-autosomal 1) (Saxena *et al.*, 1996; Yen *et al.*, 1997). It has been hypothesized that Y-linked *DAZ* perhaps originated from the translocation and amplification of this ancestral autosomal gene. Mice also possess a single autosomal *Dazl1* gene, although it appears that murine evolution did not encounter similar translocation and amplification onto the Y chromosome (Cooke *et al.*, 1996). Loss of *Dazl1* in knockout mice has been shown to result in a reduced number of germ cells as well as the complete elimination of gamete production. This phenomenon is experienced in both males and females and strongly suggests that *Dazl1* is required for gametogenesis (Ruggiu *et al.*, 1997).

The role of Y-linked *DAZ* in spermatogenesis, on the other hand, remains unclear. A number of investigators initially considered Y-linked *DAZ* to be the best candidate for *AZF* because of its deletion in many azoospermic and oligozoospermic men. The most convincing evidence in support of this notion came from studies involving *boule*, a gene isolated in Drosophila displaying homology to the DAZ family. It has been demonstrated that mutations in *boule* result in spermatocyte arrest at the G2/M transition, manifesting as complete azoospermia (Castrillon *et al.*, 1993; Eberhart *et al.*, 1996).

The difficulty in concluding Y-linked *DAZ* to be *AZF* lies in the fact that individuals devoid of *DAZ* exhibit a variety of phenotypes spanning the entire infertility spectrum from azoospermia to rare cases of fertility (Vogt *et al.*, 1996; Pryor *et al.*, 1997). This therefore suggests that perhaps the gene is not essential for the completion of normal spermatogenesis. Subsequent studies into the sequence of *boule* revealed it to be more closely related to autosomal *DAZL1* and *Dazl1* than it is to Y-linked *DAZ* (Cooke *et al.*, 1996; Shan *et al.*, 1996; Yen *et al.*, 1997). One of the reasons for this notion comes from the fact that a unique domain of 130 bp is found only in *DAZL1*, *Dazl1* and *boule* but not Y-linked *DAZ*.

It has also been observed that the majority of infertile males lacking Y-linked *DAZ* carry rather large deletions of the Y chromosome that can often stretch from *AZFc* to *AZFa* (Vogt *et al.*, 1996). Therefore, because no point mutations of the *DAZ* genes have been identified in any of the infertile men studied, it cannot be determined whether the phenotypes of infertility observed are a result of the absence of Y-linked *DAZ* or by independent mutations or deletions of neighbouring genes in the *AZFc* region.

Yen and colleagues also demonstrated a high degree of polymorphism in the *DAZ* repeat region of human Y-linked *DAZ* genes (Yen *et al*., 1997). In this experiment, five *DAZ* cDNA clones were isolated from a testis cDNA library composed of RNA pooled from four individuals displaying normal spermatogenesis. Each cDNA clone differed not only in the number of *DAZ* repeats but also in the order of the repeat units. It has been suggested that this variation could potentially result in each *DAZ* isoform possessing different properties, for example their affinity for RNA. Since *DAZ* copy number and *DAZ* repeat varies amongst individuals, the question arises whether such factors have any profound affect on sperm count and thus fertility.

In contrast, a recent transgene study conducted by Slee and coworkers seems to partially refute this dismissal of Y-linked *DAZ*'s role in spermatogenesis (Slee *et al*., 1999). In this case, a human YAC of 225 kb containing a Y-linked *DAZ* gene was introduced into a *Dazl1* knockout mouse (termed *Dazl1*$^{-/-}$). *Dazl1*$^{-/-}$ mice are normally characterized by severe germ cell depletion and meiotic failure and though the transgenic mice remained infertile, a partial and variable salvage of the mutant phenotype was observed with an elevated number of germ cells surviving up to the pachytene stage of meiosis. However, some caution must be exercised when interpreting this data as the *DAZ* transgene that was introduced lacked exon-1 and the experiment did not, therefore, conclusively establish the function of the full *DAZ* gene product. It does nevertheless suggest that at least part of the function of *DAZL1* has been retained by human Y-linked *DAZ*.

Other genes mapped to the *AZFc* region of Yq include *CDY1* (chromodomain Y1) *BPY2* (basic protein Y2), *PRY* (PTA-BL related Y) and *TTY2* (testis transcript Y2) (Lahn and Page, 1997; Yen *et al.*, 1997). Though the functions of these genes have yet to be elucidated, they do share common characteristics such as existing in multiple copies on the Y chromosome, they are expressed uniquely in the testis and are Y specific (Lahn and Page, 1997). Restriction mapping has also identified three *PRY* and *TTY2* genes in the proximal portion of *AZFc*, which are thus are unlikely to be involved in the spermatogenic disruption experienced by patients with deletions restricted to *DAZ* (Yen, 1998). Two *CDY1* genes map in the *AZFc* region, one within the *DAZ* cluster and the other at the distal end. Because at least one *CDY1* copy is invariably absent in individuals with *DAZ* deletions, the general consensus is that *CDY1* is an *AZFc* candidate gene; however, deletions specifically removing this gene should be identified before confirming this hypothesis.

## The genetic association between CF and CBAVD

It has been described that of the 25% or so of infertile patients exhibiting azoospermia, 30% possess an obstructive process (obstructive azoospermia) whilst the remainder exhibit primary testicular malfunction (non-obstructive azoospermia) (Patrizio and Leonard, 2000). Of those diagnosed with obstructive azoospermia, 25% present CBAVD. The prevalence of this condition is ~2% of all infertile males and ~16 000 individuals in the USA alone are subject to this circumstance (Patrizio and Leonard, 2000).

CBAVD is a disorder characterized by bilateral regression of variable sections of the epididymis, vas deferens and, in ~80% of cases, deficiency of the seminal vesicles (Patrizio and Leonard, 2000). A similar condition, congenital unilateral absence of the vas deferens (CUAVD) involves aplasia of only one side of the vas deferens. Because of the remarkable similarities between these anatomical properties and that observed in men with CF, it was proposed that these seemingly discrete disorders were in fact derived from the same genetic origin (Holsclaw *et al.*, 1971).

The validity of this hypothesis was supported when mutations in the CF transmembrane conductance regulator (*CFTR*) gene (Kerem *et al.*, 1989; Riordan *et al.*, 1989; Rommens *et al.*, 1989) were detected in both patients with CF as well as infertile men with CBAVD (Dumur *et al.*, 1990). The fact that the majority of men with CBAVD also present sub-clinical CF symptoms, such as mild elevations of sweat chloride concentrations, nasal polyps and chronic sinusitis, has further reinforced the idea of a genetic nexus between the two disorders. It has also been determined that ~70% of CBAVD patients exhibit at least one mild CF symptom (Durieu *et al.*, 1995), endorsing the idea that CBAVD is in reality a mild or incomplete variety of CF (Hargreave, 2000).

Males exhibiting CBAVD generally undergo normal or only slightly reduced spermatogenesis (Silber *et al.*, 1990) and can therefore benefit from assisted reproduction technologies such as epididymal sperm aspiration, IVF and, more recently, ICSI. Indeed many pregnancies leading to live births have been reported (Patrizio *et al.*, 1988; Tournaye *et al.*, 1994; Silber *et al.*, 1995) and the success of the aforementioned methods in combination with screening techniques such as pre-implantation genetic

**Table III.** Common CFTR mutations identified in patients with congenital bilateral absence of the vas deferens and their effects

| Mutation | Effect |
|---|---|
| G542X | Nonsense mutation—stop codon at position 542 |
| R553X | Nonsense mutation—stop codon at position 553 |
| W1282X | Nonsense mutation—stop codon at position 1282 |
| N1303K | Missense mutation |
| 1717–1G→A | Splicing error |
| 2184delA | Frameshift mutation |
| R1161X | Nonsense mutation—stop codon at position 1161 |

(Reproduced with permission from McElreavey, K. (ed) *The genetic basis of male infertility*. Mutations of the cystic fibrosis gene and congenital absence of the vas deferens, Patrizio, P. and Leonard, D.G.B. (2000) 175–186).

diagnosis provides CBAVD individuals with hope of a reproductive future.

***Confirmation of the genetic relationship between CF and CBAVD***

CF is a fatal autosomal recessive disorder and the most common genetic disease amongst Caucasians with an incidence of 1 in 2000–2500 live births (Hargreave, 2000). Symptoms of CF include obstructive pulmonary disease, pancreatic exocrine deficiency, gastrointestinal obstruction, elevated concentrations of electrolytes in sweat, and in males, absence of the vas deferens resulting in infertility (Patrizio and Leonard, 2000). However, the severity of the disease cannot be accurately foretold by genotype alone, as the range of presenting symptoms associated with CF is quite vast (Estivill, 1996).

The gene responsible for CF was first identified in 1989 (Rommens *et al.*, 1989) and was shown to map to the long arm of chromosome 7 (7q31.2) (Kerem *et al.*, 1989; Riordan *et al.*, 1989). The *CFTR* gene stretches ~230 kb and consists of 27 exons with the multidomain glycoprotein encoded comprising 1480 amino acids and acts as a low voltage, cAMP-regulated chloride ion channel (Patrizio and Leonard, 2000). The CFTR protein is present in concentrated levels in the apex membranes of various epithelial cells within the organs typically affected by CF (Patrizio and Leonard, 2000) and with respect to fertility, has been demonstrated to influence the formation of the ejaculatory duct, seminal vesicles, vas deferens and distal two-thirds of the epididymis (Hargreave, 2000).

Conclusive evidence establishing a shared genetic origin between CF and CBAVD arose through the work of Dumur and colleagues who in a genetic survey of 17 patients with CBAVD found seven of them to possess the {utri}F508 CF mutation (Dumur *et al*., 1990). Two other patients, though devoid of any detectable mutation, also exhibited elevated sweat chloride concentrations indicative of CF. These early findings were universally endorsed by subsequent studies (Anguiano *et al.*, 1992; Patrizio *et al.*, 1993). Moreover, CUAVD was also identified to be associated with mutations in the *CFTR* gene (Mickle *et al.*, 1995; Jezequel *et al.*, 1996)

There are currently >700 published mutations in the CFTR gene that are related to the disease, as well as 68 coding region and 55 non-coding region polymorphisms that are not (Patrizio and Leonard, 2000). The incidence of mutated *CFTR* genes in infertile men owing to CBAVD has been found to be 20 times greater than the carrier frequency detected in the general population (Patrizio and Leonard, 2000). Mutations identified in the CFTR gene, based on molecular mechanisms, can be divided into four categories:

(i) Class I mutations result in defective production of the CFTR protein through presentation of translational termination signals in the CFTR mRNA. Examples include insertions, deletions, missense mutations or splice site variants and as a result very little, if any, CFTR protein is produced;

(ii) Class II mutations involve defective processing of the CFTR protein which becomes ensnared in the endoplasmic reticulum, preventing it from ever reaching the cell surface;

(iii) Class III mutations alter the regulation of the CFTR protein function leading to decreased chloride conductance;

(iv) Class IV mutations promote malfunction in the chloride ion conductance function of the CFTR protein.

CF phenotypes can be dichotomized into severe and mild forms of the disease with the severity of the symptoms depending, by and large, on the amount of CFTR protein expressed on the cell surface. Severe CF phenotypes involve mutations that severely deplete the amount of CFTR protein whilst milder phenotypes are associated with mutations that disrupt the function or amount of CFTR to a lesser degree. CBAVD patients normally possess either a severe and a mild mutation of the *CFTR* gene, or two mild mutations simultaneously. The most frequent mutations associated with CBAVD belong to Class II, and promote defective processing of the CFTR protein. This can cause phenotypes such as pancreatic insufficiency when acting in concert with another severe mutation (Patrizio and Leonard, 2000). The most prevalent of this group is {utri}F508 which is responsible for ~70% of the CF mutations in patients. Other common CBAVD-related mutations can be found in Table III.

Amongst CBAVD patients, the missense mutation R117H located in exon 4 has also been associated with inappropriate CFTR levels arising as a result of abnormalities in the splicing efficiency of exon 9. The area of variation involves a polyprimidine tract within the acceptor splice site of intron 8 (IVS8) of the *CFTR* gene. Three variants: IVS8-5T, IVS8-7T and IVS8-9T have been reported and correspond to 5, 7 and 9 thymidines respectively. The length of the T-tract influences the splicing efficiency of exon 9 and therefore the proportion of normal *CFTR* mRNA. The longer the tract the greater the splicing efficiency, thus the 9 thymidine tract is most efficient in contrast to the 5 T-tract which permits only 8–10% of *CFTR* to be concluded (Patrizio and Leonard, 2000). Since CFTR protein transcribed from mRNA devoid of exon 9 does not function as a cAMP-activated chloride channel, the 5T variant's low splicing efficiency induces the phenotypic affect of the R117H mutation on the same allele. Recent screening for the 5T, 7T and 9T variants in the polythymidine tract of IVS8 demonstrated a 5- to 6-fold increase in the frequency of the 5T allele among CBAVD chromosomes with the presence of the 5T variant reported to dramatically diminish the levels of functional CFTR (Patrizio and Leonard, 2000). The total amount of CFTR expressed may be the difference between the manifestation of CF or CBAVD and, as Table IV demonstrates, the severe depletion of CFTR levels results in the CF phenotype being exhibited whilst mild depletion results in the development of CBAVD. As the majority of

**Table IV.** Quantification of functional CFTR in relation to the severity of the phenotype

| Amount of functional CFTR | Affected organs | Phenotype |
|---|---|---|
| 0–10 | Lungs, sweat glands, pancreas, vas deferens | CF |
| 11–50 | Vas deferens | CBAVD |
| 51–100 | None | Normal |

(Permission as Table III)
CF = cystic fibrosis; CBAVD = congenital bilateral absence of the vas deferens

CBAVD men possess only one detectable mutation, sufficient functional CFTR may be available to alleviate the symptoms and thus present only a mild form of CF in such cases.

From a pathological perspective, the most likely explanation for the absence of the vas deferens and for variable epididymal length experienced by CBAVD patients involves the progressive obstruction of the deferent ductal system arising as a result of excessively viscous mucus secretions in the epididymal lumen. This surplus build-up of secretions has been thought to induce damage and subsequent atrophy and deterioration of the aforementioned structures. Insufficient levels of CFTR means sodium and water reabsorption from the epididymal lumen significantly exceeds the limited levels of chloride secretion, and consequently the epididymal fluid thickens, thus hindering the correct development of the most distal segments of the epididymis in addition to the remaining derivatives of the Wolffian ducts, vas deferens and seminal vesicles. Such abnormalities cumulatively contribute to infertility.

## Insights into male infertility from mouse studies into spermatogenic function

Insights into the mechanisms of human male infertility can be established by investigating the associated mechanisms in model organisms. For example, recent murine studies into apoptosis regulation during spermatogenesis have identified a number of factors including cAMP response element modulator and protamine, which are critical in this process (Fimia *et al.*, 2001; review). Other factors regulating apoptosis during spermatogenesis include the *Bcl-2* gene family. Furthermore, it has also been established that interaction between the c-kit tyrosine kinase receptor and its complimentary ligand, SCF, influence cell proliferation, survival, adhesion and migration during the early stages of murine spermatogenesis. Whilst these studies specifically demonstrate the importance of these respective systems in murine fertility, it is believed that findings from such investigations may translate to understanding similar processes relating to human fertility/infertility.

### *Apoptosis regulation during spermatogenesis: insights into the relationship between the Bcl-2 family and male infertility*

Proteins of the Bcl-2 family provide one signalling pathway that appears imperative in maintaining male germ cell homeostasis, thereby acting as key regulators of apoptosis. This concerted programme of cellular attrition is vital for the removal of surplus, aged, defective or damaged cells and is a crucial component of normal animal development, especially with respect to spermatogenesis. All members of the Bcl-2 family possess at least one of four conserved motifs referred to as Bcl-2 homology domains (BH1–BH4). This family can be dichotomized into opposing factions with the pro-survival wing inhibiting apoptosis in the face of a plethora of insults including cytokine deprivation and irradiation. Members of this anti-apoptotic group contain at least BH1 and BH2; however, those that most closely resemble the product of the prototypical *Bcl-2* gene possess all four domains (Adams and Cory, 1998).

Within the opposing pro-apoptotic faction, two subgroups that differ markedly in their homology to Bcl-2 exist. The Bax subfamily is made up of three homologues (Bax, Bak and Bok) that contain BH1, BH2 and BH3 and resembles Bcl-2 most closely. In contrast, the remaining known pro-apoptotic proteins fall within a group associated with the Bcl-2 protein only by their possession of the central short (9–16 amino acid) BH3 domain, and thus are referred to as the BH3 subfamily. The BH3 domain has been demonstrated to be essential for the pro-apoptotic function of these proteins (Conradt and Horvitz, 1998). Furthermore, it has been observed that this subfamily is quite unrelated to any other known protein and even within their ranks only Bik and Blk share similarity.

It has also been suggested that because members of these opposing factions associate and seemingly titrate one another's function, their relative abundance in a particular cell type may well determine its threshold for apoptosis (Oltvai *et al.*, 1993).

#### *Apoptotic regulation by the Bcl-2 family during murine spermatogenesis*

Extensive apoptosis of pre-meiotic germ cells is observed during the preliminary round of sperm production and in mice generally lasts up to 5 weeks, peaking at ~2 weeks pp (Rodriguez *et al.*, 1997; Wang *et al.*, 1998). It has been postulated that such a period of concerted cellular attrition, perhaps mediated by high levels of the pro-apoptosis protein Bax, is required in order to adjust the number of germ cells to match that which can be maintained by the available supporting Sertoli cells (Print *et al.*, 1998).

The significance of this early germ cell attrition on the subsequent production of spermatozoa has been demonstrated using genetically modified mice. For example, inhibition of apoptosis through the loss of endogenous Bax, a pro-apoptotic member of the Bcl-2 family, results in the accumulation of pre-meiotic cells and failure of both the first wave of spermatogenesis and subsequent adult spermatogenesis (Knudson *et al.*, 1995). A similar result is also observed in mice expressing transgenic *Bcl-2* or *Bcl-$x_L$* (Furuchi *et al.*, 1996; Rodriguez *et al.*, 1997).

Either an increase in pro-apoptotic or anti-apoptotic proteins can contribute to an imbalance in apoptosis regulation. In the case of Bax deficiency, the germ cell to Sertoli cell ratio critical to spermatogenesis is disrupted. This imbalance results in the pro-survival proteins, e.g. Bcl-2, having a decreased number of pro-apoptosis antagonists to oppose their activity, thus allowing pre-meiotic cells to proliferate beyond normal acceptable thresholds (Knudson *et al.*, 1995). Studies have also demonstrated that transgenic expression of Bcl-2 or Bcl-$x_L$ leads to elevated levels of these pro-survival proteins that exceed tolerable parameters (Furuchi *et al.*, 1996; Rodriguez *et al.*, 1997).

In contrast to *Bcl-2* or *Bcl-$x_L$* transgenic mice and *Bax* knockout mice, all of which exhibit spermatogenic failure from the outset, null mutation of another pro-survival apoptotic protein, Bcl-w, appears to have no effect upon the first wave of spermatogenesis (Print *et al.* 1998). The testes of 2 week old *bcl-$w^{\triangle/\triangle}$* mice appear largely unaffected by the loss of this gene and display normal mass and histology, despite wild-type mice of a comparable age expressing copious amounts of Bcl-w in the Sertoli and germ cells (Print *et al.*, 1998). The rationale behind this observation is that the presence of alternative pro-survival proteins, for example Bcl-$x_{L,}$ compensates for this deficiency. Studies have suggested that Bcl-$x_L$ is abundantly expressed in the testis from 1–3 weeks pp and accommodates the absence of Bcl-w during this formative period, effectively maintaining equilibrium between cell proliferation and apoptosis (Rodriguez *et al.*, 1997).

Adult spermatogenesis, however, appears intrinsically dependent on the activity of Bcl-w (Print *et al.*, 1998). Young adult *bcl-$w^{\triangle/\triangle}$* mice gradually experience degeneration of Sertoli cells and germ cells as the number of pro-apoptotic proteins begins to exceed the number of pro-survival proteins. Indeed, by 8 weeks of age the number of apoptotic cells observed was five times the normal level and as a consequence, the testes were observed to have lost 70% of their mass (Print *et al.*, 1998). Sertoli cells in such mutants succumb as expression of the pro-death protein Bak in the absence of Bcl-w leads to a Bak-induced demise (Krajewski *et al.*, 1996). Similarly, it has been suggested that the deterioration of germ cells is perhaps a result of Bax, a pro-apoptotic protein that despite being transcribed at relatively low levels is probably still sufficient to promote cell death in the absence of any antagonist (Rodriguez *et al.*, 1997). No evidence has been presented to suggest that any of the other pro-survival proteins detected in the testis compensates for the lack of Bcl-w. Bcl-2 is present only in mature sperm and not in the seminiferous epithelium (Hockenbery *et al.*, 1991) whilst Mcl-1 (a further pro-survival member of the Bcl-2 subfamily) is restricted to Leydig cells (Krajewski *et al.*, 1995). Furthermore, Bcl-$x_L$ is localized to spermatocytes and spermatids and even then is expressed at very low levels (Krajewski *et al.*, 1996; Rodriguez *et al.*, 1997).

Germ cell reduction in adult *bcl-$w^{\triangle/\triangle}$* mice may also be attributed to extracellular effects such as the loss of Sertoli cells. Evidence to suggest the relationship between Sertoli cell demise and germ cell death arose from studies involving the experimental reduction of Sertoli cells in the testes of immature rats. The validity of this nexus was established after a proportionate decrease in the number of round spermatids was observed in the adult animal (Orth, 1982).

### *Stem cell factor and c-kit tyrosine kinase receptor interactions in the mammalian testis*

The location of the Sertoli cell in the seminiferous epithelium, i.e. extending from the innermost layer of the basement membrane lining the seminiferous tubule through to the lumen, is unique in that it permits communication with all germ cell generations, myoid cells and through its base, the cells of the interstitium (Jegou *et al.*, 2000). Due to their central position, Sertoli cells can effectively provide the necessary chemical and physical signals required for division, differentiation, migration and metabolism of neighbouring germ cells. The intricate process whereby spermatogonial stem cells differentiate into haploid spermatozoa is particularly dependent on the influence of adjacent Sertoli cells. Moreover, spermatogonial stem cells are essentially the only self-renewing cell type in the adult capable of affording a genetic contribution to the next generation (Brinster and Zimmermann, 1994), and thus through germ-cell transplantation, stem cells isolated from testes of donor mice have been shown to repopulate sterile recipient testes when introduced into seminiferous tubules. Subsequent donor cell spermatogenesis generates morphologically normal mature spermatozoa (Brinster and Zimmermann, 1994). The possibility of genetic modification of spermatogonial stem cells prior to transplantation also has the potential to produce a large number of transgenic progeny.

One particular example of the interaction between Sertoli cells and germ cells is the system involving the *c-kit* tyrosine kinase receptor (Yarden *et al.*, 1987; Chabot *et al.*, 1988) and its ligand, SCF (Anderson *et al.*, 1990; Copeland *et al.*, 1990; Martin *et al.*, 1990; Zsebo *et al.*, 1990). This has been demonstrated to mediate communication between these cells during early spermatogenesis (Figure 2) with the subsequent signalling cascade arising from this interface implicated in primordial germ cell migration, spermatogonial adhesion and proliferation as well as inhibition of apoptosis in the testes (Loveland and Schlatt, 1997). Animal models possessing defects in the genes encoding either of these proteins have been associated with reduced fertility or even a complete absence of germ cells in the testes (Loveland and Schlatt, 1997).

The *c-kit* gene has been determined to exist within the *White-spotting* (*W*) locus located on the proximal long arm of chromosome 5 in humans and chromosome 4 in mice (Yarden *et al.*, 1987; Chabot *et al.*, 1988). The 21-exon proto-oncogene encodes a tyrosine kinase receptor (Giebel *et al.*, 1992; Vandenbark *et al.*, 1992) that binds its ligand, SCF (Figure 2). The c-kit receptor is anchored to the cell membrane by a 23 amino acid hydrophobic domain and a larger 433-residue region comprising a catalytic kinase domain, a kinase insert and an ATP-binding site. Interaction with the SCF ligand at the extracellular domain prompts receptor dimerization resulting in autophosphorylation of the c-kit subunits (Mauduit, 1999). Autophosphorylation initiates intracellular signalling pathways that may be involved in cell proliferation, survival and adhesion (Loveland and Schlatt, 1997; Figure 2).

Expression of *c-kit* mRNA can be found in Leydig cells, spermatogonia, primary spermatocytes and round spermatids, though the last of these testicular cells contains mRNA encoding a truncated c-kit protein consisting of only part of the intracellular domain (Loveland and Schlatt, 1997).

The corresponding ligand for c-kit is SCF, an integral membrane glycoprotein encoded by nine exons (Martin *et al.*, 1990; Brannan *et al.*, 1992) within the *Steel* (*Sl*) locus located on chromosome 12 in humans and chromosome 10 in mice (Anderson *et al.*, 1990; Copeland *et al.*, 1990; Martin *et al.*, 1990; Zsebo *et al.*, 1990). Mutations within this locus have been demonstrated to produce *SCF* mRNAs with abnormal expression patterns as well as generating proteins with irregular or absent cytoplasmic tails (Huang *et al.*, 1993; Bedell *et al.*, 1995).

As a result of alternative splicing, SCF is synthesized as one of two distinct isoforms: a soluble protein (SCFs) and a membrane-bound alternative (SCFm). The former protein is preferentially spliced to include exon 6, which has been demonstrated to encode

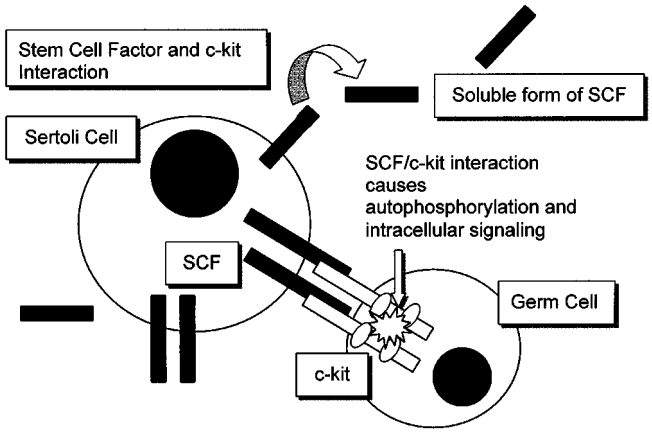


**Figure 2.** Stem cell factor interacts with its target c-kit receptor protein as a dimer. The subsequent autophosphorylation effects signal transduction influencing cell proliferation, survival and adhesion. (Reproduced by permission of the Society for Endocrinology; Loveland and Schlatt, 1997)

an amino acid sequence of 84 bp that is readily cleaved by proteases on the surface of cells *in vitro*, leading to a soluble form of the protein (Huang *et al.*, 1992). The latter isoform is devoid of this exon and thus somewhat resistant to proteolytic cleavage, rendering it more stable at the cell surface interface. Notwithstanding this resistance, an alternative form of the soluble SCF protein can also be generated through cleavage of a site found within sequences encoded by exon 7. However, the cleavage rate through this route is substantially slower than that induced by the corresponding sequences encoded by exon 6.

SCFm predominates in the newborn mouse testis, but this situation reverses in the adult where the soluble variety of the protein is prevalent (Manova *et al.*, 1993). Whilst both the soluble and membrane-anchored forms of SCF produced by the Sertoli cells have the potential to interact with the c-kit receptor expressed on adjacent spermatogonia, with the latter form communicating through direct cellular contact, dialogue with distal targets such as c-kit receptors on Leydig cells in the interstitium may be achieved when SCFs is released basally (Loveland and Schlatt, 1997).

*Expression and function in gonadal development during embryogenesis*

mRNA encoding c-kit can be detected in PGCs at ~7 days post coitum (pc) and remains detectable at 12.5 days pc, at which stage the PGCs have already migrated to the gonadal ridge in both males and females (Orr-Urtreger *et al.*, 1990). It is suggested that during this period the interaction between membrane-bound SCF expressed on Sertoli cell precursors in the embryonic ridge and c-kit expressed on the surface of adjacent PGCs is essential for normal PGC proliferation and migration. Evidence supporting this notion arises in the form of *SCF* mRNA being detected by in-situ hybridization, starting at 9 days pc along the migratory pathway and in the genital ridge by 12.5 days pc (Matsui *et al.*, 1990).

A rapid decline in the number of PGCs during embryogenesis is also observed in both *Sl/Sl* and *W/W* homozygotes. Though normal numbers of PGCs are present at 8.5 days pc, prompt deterioration results in only 2% of the normal level being accounted for by day 12.5 pc (Loveland and Schlatt, 1997). By day 14 pc, *Sl/Sl* mice are completely devoid of germ cells due to the PGCs having not made the journey from the hind gut to the gonadal ridge, while only some migration is observed to occur in *W/W* individuals. The exact mechanisms underlying this failed migration are not fully understood, though it has been suggested that the absence of c-kit/SCF interaction results in the loss of signals for guidance, survival and proliferation (Print and Loveland, 2000).

SCF has also been demonstrated to be a requirement during the first wave of spermatogenesis. During the first 10 days pp, the level of SCF expression in the murine testis becomes markedly elevated. Seminiferous tubules undergoing the first wave of spermatogenesis were conspicuously devoid of differentiating type A spermatogonia if they had been treated with ACK2, a monoclonal antibody that blocks binding of SCF to c-kit (Yoshinaga *et al.*, 1991). All other cells in these animals, however, appeared unaffected. Furthermore, the frequency of apoptosis in spermatogonia and spermatocytes was also observed to have increased in these mice, suggesting that SCF affords a survival signal in these cell types.

In adult mice, in-vivo experiments involving inhibition of c-kit/SCF interactions through administration of the ACK2 antibody demonstrated an increased incidence of apoptosis in spermatogonia and spermatocytes, emphasizing the role of this system in the regulation of germ cell apoptosis (Packer *et al.*, 1995). Similarly, in an in-vitro study, all germ cell types appeared protected from apoptosis by the introduction of exogenous SCF (Yan *et al.*, 2000). Furthermore, injection of SCF directly into the testis was shown to accelerate the recovery of germ cell populations elicited by Sertoli cell depletion (Allard *et al.*, 1996).

## Conclusion

It has been established that chromosomal abnormalities, both affecting the sex chromosomes and the autosomes, occur more frequently in infertile male populations compared with newborns. A variety of such irregularities have been described including reciprocal translocations, Robertsonian translocations, paracentric inversions, marker chromosomes and aneuploidy, all of which have been demonstrated to detrimentally affect the ability to procreate. Klinefelter's syndrome, the most common chromosomal disorder associated with male infertility, typically occurs as a consequence of a 47,XXY chromosome complement although similar mosaicism has also been reported to manifest the disorder. The activity of an additional surplus X chromosome supposedly inhibits the survival of male germ cells. However, recent evidence involving the identification of 24,XY hyperhaploids from Klinefelter patients suggests a meiotic capacity. Various genetic disorders manifesting as male infertility involving impaired gonadotrophin activity have also been widely reported with such conditions more specifically concerning disruptions in GnRH secretion and action, pituitary LH and FSH secretion and action, and pituitary development disorders.

Microdeletions of the Y chromosome have also been recognized as having a possible influence on spermatogenesis, with several surveys of infertile men revealing a number of azoospermic and oligozoospermic patients exhibiting this absence of genetic material. Researchers have identified four separate areas on the long arm of Y, termed *AZFa*, *AZFb*, *AZFc* and recently *AZFd* that

contain numerous genes and gene families considered candidates for azoospermia. Examples of these include *RBMY*, *DAZ* and *USP9Y*, the first two of which are exclusively expressed in the testes and are believed to have RNA-binding activity whilst the latter is widely expressed and is homologous to the Drosophila developmental gene fats facet (*faf*), a de-ubiquinating enzyme.

CF, the most common genetic disorder in Caucasian populations, and CBAVD, a condition experienced by 25% of cases displaying obstructive azoospermia, are believed to share a common genetic origin. Mutations in the *CFTR* gene are responsible for both situations arising, though the severity of the CFTR deficiency determines the phenotype. CF manifests as a result of severe CFTR shortage whilst CBAVD is experienced when this absence is less acute. Thus, it is held that CBAVD is merely a subtle variety of CF.

Infertility studies involving mouse models have also provided unique insights into the mechanisms behind spermatogenesis. The regulation of apoptosis, for example, especially during the first wave of spermatogenesis, is vital to reproductive viability in the mammalian system. Recent studies in mice have shown the Bcl-2 family to be key mediators of this process, with opposing factions of the cohort exhibiting pro-survival and pro-apoptosis activity respectively. An imbalance in the equilibrium between the rival Bcl-2 groups disrupts the delicate Sertoli cell to germ cell ratio critical to spermatogenesis. The pro-survival protein, Bcl-w, in particular, has been demonstrated to be vital in adult spermatogenesis but otherwise redundant for normal development. The interaction between the tyrosine kinase receptor, c-kit, and its ligand, SCF, which are expressed on germ cells and Sertoli cells respectively, has also been demonstrated to mediate PGC migration, spermatogonial adhesion, proliferation and apoptosis inhibition in the mouse. Indeed, defects in either of the genes encoding these proteins have been associated with infertility and have provided evidence to support the importance of this interaction in spermatogenesis. Therefore, the importance of animal models in furthering understanding of human infertility cannot be understated as such findings are often translated to the human system.

## References


Adams, J.M. and Cory, S. (1998) The Bcl-2 protein family: arbiters of cell survival. *Science*, **281**, 1322–1326.

Allard, E.K., Blanchard, K.T. and Boekelheide, K. (1996) Exogenous stem cell factor (SCF) compensates for altered endogenous SCF expression in 2,5 hexanedione-induced testicular atrophy in rats. *Biol. Reprod.*, **55**, 185–193.

Anderson, D.M., Lyman, S.D., Baird, A., Wignall, J.M., Eisenman, J., Rauch, C., March, C.J., Boswell, H.S., Gimpel, S.D., Cosman, D. *et al.* (1990) Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell*, **63**, 235–243.

Anguiano, A., Oates, R.D. and Amos, J.A. (1992) Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. *J. Am. Med. Assoc.*, **267**, 1794–1797.

Arbizu, T., Santamaria, J., Gomez, J.M., Quilez, A. and Serra, J.P. (1983) A family with adult spinal and bulbar muscular atrophy, X-linked inheritance and associated testicular failure. *J. Neurol. Sci.*, **59**, 371–82.

Ballabio, A., Bardoni, B., Carrozzo, R., Andria, G., Bick, D., Campbell, L., Hamel, B., Ferguson-Smith, M.A., Gimelli, G., Fraccaro, M. *et al.* (1989) Contiguous gene syndromes due to deletions in the distal short arm of the human X chromosome. *Proc. Natl Acad. Sci. USA*, **86**, 10001–10005.

Bedell, M.A., Brannan, C.I., Evans, E.P., Copeland, N.G., Jenkins, N.A. and Donovan, P.J. (1995) DNA rearrangements located over 100 kb 5<prime> of the Steel (Sl)-coding region in Steel-panda and Steel-contrasted mice deregulate Sl expression and cause female sterility by disrupting ovarian follicle development. *Genes Dev.*, **9**, 455–470.

Bhasin, S., Mallidis, C. and Ma, K. (2000) The genetic basis of infertility in men. *Baillières Best Pract. Res. Clin. Endocrinol. Metab.*, **14**, 363–388.

Bielanska, M., Tan, S.L. and Ao, A. (2000) Fluorescence in-situ hybridization of sex chromosomes in spermatozoa and spare preimplantation embryos of a Klinefelter 46,XY/47,XXY male. *Hum. Reprod.*, **15**, 440–444.

Brannan, C.I., Bedell, M.A., Resnick, J.L., Eppig, J.J., Handel, M.A., Williams, D.E., Lyman, S.D., Donovan, P.J., Jenkins, N.A. and Copeland, N.G. (1992) Developmental abnormalities in Steel17H mice result from a splicing defect in the steel factor cytoplasmic tail. *Genes Dev.*, **6**, 1832–1842.

Brinster, R.L. and Zimmermann, J.W. (1994) Spermatogenesis following male germ-cell transplantation. *Proc. Natl Acad. Sci. USA*, **91**, 11298–11302.

Brown, G.M., Furlong, R.A., Sargent, C.A., Erickson, R.P., Longepied, G., Mitchell, M., Jones, M.H., Hargreave, T.B., Cooke, H.J. and Affara, N.A. (1998) Characterisation of the coding sequence and fine mapping of the human DFFRY gene and comparative expression analysis and mapping to the Sxrb interval of the mouse Y chromosome of the Dffry gene. *Hum. Mol. Genet.*, **7**, 97–107.

Calvo, L., Vantman, D., Banks, S.M., Tezon, J., Koukoulis, G.N., Dennison, L. and Sherins, R.J. (1989) Follicular fluid-induced acrosome reaction distinguishes a subgroup of men with unexplained infertility not identified by semen analysis. *Fertil. Steril.*, **52**, 1048–1054.

Cassidy, S.B. and Schwartz, S. (1998) Prader–Willi and Angelman Syndromes. Disorders of genomic imprinting. *Medicine*, **77**, 140–151.

Castrillon, D.H., Gonczy, P., Alexander, S., Rawson, R., Eberhart, C.G., Viswanathan, S., DiNardo, S. and Wasserman, S.A. (1993) Toward a molecular genetic analysis of spermatogenesis in *Drosophila melanogaster*: characterization of male-sterile mutants generated by single P element mutagenesis. *Genetics*, **135**, 489–505.

Chabot, B., Stephenson, D.A., Chapman, V.M., Besmer, P. and Bernstein, A. (1988) The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature*, **335**, 88–89.

Chai, N.N., Salido, E.C. and Yen, P.H. (1997) Multiple functional copies of the RBM gene family, a spermatogenesis candidate on the human Y chromosome. *Genomics*, **45**, 355–361.

Chai, N.N., Zhou, H., Hernandez, J., Najmabadi, H., Bhasin, S. and Yen, P.H. (1998) Structure and organization of the RBMY genes on the human Y chromosome: transposition and amplification of an ancestral autosomal hnRNPG gene. *Genomics*, **49**, 283–289.

Chandley, A.C. (1975) Human meiotic studies. In Emery, A.E.H. (ed.) *Modern Trends in Human Genetics.* Butterworths, London, UK, pp. 31–82.

Chandley, A.C. (1979) The chromosomal basis of human infertility. *Br. Med. Bull.*, **35**, 181–186.

Chevret, E., Rousseaux, S., Monteil, M., Usson, Y., Cozzi, J., Pelletier, R. and Sele, B. (1996) Increased incidence of hyperhaploid 24,XY spermatozoa detected by three-colour FISH in a 46,XY/47,XXY male. *Hum. Genet.*, **97**, 171–175.

Conradt, B. and Horvitz, H.R. (1998) The *C. elegans* protein EGL-1 is required for programmed cell death and interacts with the Bcl-2-like protein CED-9. *Cell*, **93**, 519–529.

Conway, S.J., Mahadevaiah, S.K., Darling, S.M., Capel, B., Rattigan, A.M. and Burgoyne, P.S. (1994) Y353/B: a candidate multiple-copy spermiogenesis gene on the mouse Y chromosome. *Mamm. Genome*, **5**, 203–210.

Cooke, H.J., Lee, M., Kerr, S. and Ruggiu, M. (1996) A murine homologue of the human DAZ gene is autosomal and expressed only in male and female gonads. *Hum. Mol. Genet.*, **5**, 513–516.

Copeland, N.G., Gilbert, D.J., Cho, B.C., Donovan, P.J., Jenkins, N.A., Cosman, D., Anderson, D., Lyman, S.D. and Williams, D.E. (1990) Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. *Cell*, **63**, 175–183.

Cozzi, J., Chevret, E., Rousseaux, S., Pelletier, R., Benitz, V., Jalbert, H. and Sele, B. (1994) Achievement of meiosis in XXY germ cells: study of 543 sperm karyotypes from an XY/XXY mosaic patient. *Hum. Genet.*, **93**, 32–34.

Crowley, W.F. Jr, Filicori, M., Spratt, D.I. and Santoro, N.F. (1985) The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. *Recent Prog. Horm. Res.*, **41**, 473–531.

De Braekeleer, M. and Dao, T.N. (1991) Cytogenetic studies in male infertility: a review. *Hum. Reprod.*, **6**, 245–250.

Delbridge, M.L., Harry, J.L., Toder, R., O'Neill, R.J., Ma, K., Chandley, A.C. and Graves, J.A. (1997) A human candidate spermatogenesis gene,

RBM1, is conserved and amplified on the marsupial Y chromosome. *Nat. Genet.*, **15**, 131–136.

Delbridge, M.L., Ma, K., Subbarao, M.N., Cooke, H.J., Bhasin, S. and Graves, J.A. (1998) Evolution of mammalian HNRPG and its relationship with the putative azoospermia factor RBM. *Mamm. Genome*, **9**, 168–170.

Delbridge, M.L., Lingenfelter, P.A., Disteche, C.M. and Graves, J.A. (1999) The candidate spermatogenesis gene RBMY has a homologue on the human X chromosome. *Nat. Genet.*, **22**, 223–224.

Dowsing, A.T., Yong, E.L., Clark, M., McLachlan, R.I., de Kretser, D.M. and Trounson, A.O. (1999) Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. *Lancet*, **354**, 640–643.

Dumur, V., Gervais, R., Rigot, J.M., Lafitte, J.J., Manouvrier, S., Biserte, J., Mazeman, E. and Roussel, P. (1990) Abnormal distribution of CF delta F508 allele in azoospermic men with congenital aplasia of epididymis and vas deferens. *Lancet*, **336**, 512.

Durieu, I., Bey-Omar, F. and Rollet, J. (1995) Diagnostic critieria for systic fibrosis in men with congenital absence of the vas deferens. *Medicine*, **74**, 42–47.

Eberhart, C.G., Maines, J.Z. and Wasserman, S.A. (1996) Meiotic cell cycle requirement for a fly homologue of human deleted in azoospermia. *Nature*, **381**, 783–785.

Elliott, D.J., Oghene, K., Makarov, G., Makarova, O., Hargreave, T.B., Chandley, A.C., Eperon, I.C. and Cooke, H.J. (1998) Dynamic changes in the subnuclear organisation of pre-mRNA splicing proteins and RBM during human germ cell development. *J. Cell. Sci.*, **111**, 1255–1265.

Estivill, X. (1996) Complexity in a monogenic disease. *Nat. Genet.*, **12**, 348–350.

Evenson, D. and Jost, L. (2000) Sperm chromatin structure assay is useful for fertility assessment. *Methods Cell. Sci.*, **22**, 169–189.

Fimia, G.M., Morlon, A., Macho, B., De Cesare, D. and Sassone-Corsi, P. (2001) Transcriptional cascades during spermatogenesis: pivotal role of CREM and ACT. *Mol. Cell. Endocrinol.*, **179**, 17–23.

Foresta, C., Ferlin, A. and Moro, E. (2000) Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. *Hum. Mol. Genet.*, **9**, 1161–1169.

Foresta, C., Moro, E. and Ferlin, A. (2001) Y chromsome microdeletions and alterations in spermatogenesis. *Endocr. Rev.*, **22**, 226–239.

Furuchi, T., Masuko, K., Nishimune, Y., Obinata, M. and Matsui, Y. (1996) Inhibition of testicular germ cell apoptosis and differentiation in mice misexpressing Bcl-2 in spermatogonia. *Development*, **122**, 1703–1709.

Georgopoulos, N.A., Pralong, F.P., Seidman, C.E., Seidman, J.G., Crowley, W.F. Jr and Vallejo, M. (1997) Genetic heterogeneity evidenced by low incidence of KAL-1 gene mutations in sporadic cases of gonadotropin-releasing hormone deficiency. *J. Clin. Endocrinol. Metab.*, **82**, 213–217.

Giebel, L.B., Strunk, K.M., Holmes, S.A. and Spritz, R.A. (1992) Organization and nucleotide sequence of the human KIT (mast/stem cell growth factor receptor) proto-oncogene. *Oncogene*, **7**, 2207–2217.

Hardelin, J.P., Levilliers, J. and Young, J. (1993) Xp22.3 deletions in solated familial Kallman's syndrome. *J. Clin. Endocrinol. Metab.*, **76**, 827–831.

Hargreave, T.B. (2000) Genetic basis of male fertility. *Br. Med. Bull.*, **56**, 650–671.

Hockenbery, D.M., Zutter, M., Hickey, W., Nahm, M. and Korsmeyer, S.J. (1991) BCL2 protein is topographically restricted in tissues characterized by apoptotic cell death. *Proc. Natl Acad. Sci. USA*, **88**, 6961–6965.

Holsclaw, D.S., Lobel, B., Jockin, H. and Schwachman, H. (1971) Genital abnormalities in male patients with cystic fibrosis. *J. Urol*, **106**, 568–574.

Huang, E.J., Nocka, K.H., Buck, J. and Besmer, P. (1992) Differential expression and processing of two cell associated forms of the kit-ligand: KL-1 and KL-2. *Mol. Biol. Cell*, **3**, 349–362.

Huang, E.J., Manova, K., Packer, A.I., Sanchez, S., Bachvarova, R.F. and Besmer, P. (1993) The murine steel panda mutation affects kit ligand expression and growth of early ovarian follicles. *Dev. Biol.*, **157**, 100–109.

Huszar, G., Vigue, L. and Morshedi, M. (1992) Sperm creatine phosphokinase M-isoform ratios and fertilizing potential of men: a blinded study of 84 couples treated with *in vitro* fertilization. *Fertil. Steril.*, **57**, 882–888.

Jegou, B., Stephan, J.P., Cudicini, C., Gomex, E., Bauche, F., Piquet-Pellorce, C. and Touzalin, A.M. (2000) The sertoli cell–germ cell interactions and the seminiferous tubule interleukin-1 and interleukin-6 system. In McElreavey, K. (ed) *The Genetic Basis of Male Infertility.* Springer-Verlag, New York, USA, pp. 53–68.

Jezequel, P., Chauvel, B., Le Treut, A., Le Gall, J.Y., David, V., Le Lannou, D. and Blayau, M. (1996) Identification of a novel mutation in CFTR gene exon 8 (L375F) in a CUAVD phenotype. *Hum. Genet.*, **97**, 548–549.

Jones, M.H., Furlong, R.A., Burkin, H., Chalmers, I.J., Brown, G.M., Khwaja, O. and Affara, N.A. (1996) The Drosophila developmental gene fat facets has a human homologue in Xp11.4 which escapes X-inactivation and has related sequences on Yq11.2. *Hum. Mol. Genet.*, **5**, 1695–1701.

Kallman, F., Schoenfeld, W. and Barrera, S. (1944) The genetic aspect of primary eunuchoidism. *Am. J. Mental Deficiency*, **48**, 203–236.

Karsch-Mizrachi, I. and Haynes, S.R. (1993) The rb97D gene encodes a potential RNA-bindind protein required for spermatogenesis in *Drosophila. Nucleic Acids Res.*, **21**, 2229–2235.

Kaufman, M.H. (1992) *The Atlas of Mouse Development.* Academic Press Limited, San Diego, USA.

Kedes, L.H. (1979) Histone genes and histone messengers. *Ann. Rev. Biochem.*, **48**, 837–870.

Kent-First, M., Muallem, A., Shultz, J., Pryor, J., Roberts, K., Nolten, W., Meisner, L., Chandley, A., Gouchy, G., Jorgensen, L. *et al*. (1999) Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y-chromosome microdeletion detection. *Mol. Reprod., Dev.*, **53**, 27–41.

Kerem, B., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M. and Tsui, L.C. (1989) Identification of the cystic fibrosis gene: genetic analysis. *Science*, **245**, 1073–1080.

Kjessler, B. (1974) Chromosomal constituition and male reproductive failure. In Mancini, R.E. and Martini, L. (eds) *Male Fertility and Sterility.* Academic Press, New York, USA, pp. 231–247.

Knudson, C.M., Tung, K.S., Tourtellotte, W.G., Brown, G.A.and Korsmeyer, S.J. (1995) Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science*, **270**, 96–99.

Krajewski, S., Bodrug, S., Krajewska, M., Shabaik, A., Gascoyne, R., Berean, K. and Reed, J.C. (1995) Immunohistochemical analysis of Mcl-1 protein in human tissues. Differential regulation of Mcl-1 and Bcl-2 protein production suggests a unique role for Mcl-1 in control of programmed cell death *in vivo. Am. J. Pathol.*, **146**, 1309–1319.

Krajewski, S., Krajewska, M. and Reed, J.C. (1996) Immunohistochemical analysis of *in vivo* patterns of Bak expression, a proapoptotic member of the Bcl-2 protein family. *Cancer Res.*, **56**, 2849–2855.

Krausz, C. and Forti, G. (2000) Clinical aspects of male infertility. In McElreavey, K. (ed) *The Genetic Basis of Male Infertility.* Springer-Verlag, Berlin, Heidelberg, Germany, pp. 1–21.

Lahn, B.T. and Page, D.C. (1997) Functional coherence of the human Y chromosome. *Science*, **278**, 675–680.

Long, E.O. and Dawid, I.B. (1980) Repeated genes in eukaryotes. *Ann. Rev. Biochem.*, **49**, 727–764.

Loveland, K.L. and Schlatt, S. (1997) Stem cell factor and c-kit in the mammalian testis: lessons originating from Mother Nature's gene knockouts. *J. Endocrinol.*, **153**, 337–344.

Lubahn, D.B., Brown, T.R., Simental, J.A., Higgs, H.N., Migeon, C.J., Wilson, E.M. and French, F.S. (1989) Sequence of the intron/exon junctions of the coding region of the human androgen receptor gene and identification of a point mutation in a family with complete androgen insensitivity. *Proc. Natl Acad. Sci. USA*, **86**, 9534–9538.

Ma, K., Inglis, J.D., Sharkey, A., Bickmore, W.A., Hill, R.E., Prosser, E.J., Speed, R.M., Thomson, E.J., Jobling, M., Taylor, K. *et al.* (1993) A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell*, **75**, 1287–1295.

Mahadevaiah, S.K., Odorisio, T., Elliott, D.J., Rattigan, A., Szot, M., Laval, S.H., Washburn, L.L., McCarrey, J.R., Cattanach, B.M., Lovell-Badge, R. and Burgoyne, P.S. (1998) Mouse homologues of the human AZF candidate gene RBM are expressed in spermatogonia and spermatids and map to a Y chromosome deletion interval associated with a high incidence of sperm abnormalities. *Hum. Mol. Genet.*, **7**, 715–727.

Manova, K., Huang, E.J., Angeles, M., De Leon, V., Sanchez, S., Pronovost, S.M., Besmer, P. and Bachvarova, R.F. (1993) The expression pattern of the c-kit ligand in gonads of mice supports a role for the c-kit receptor in oocyte growth and in proliferation of spermatogonia. *Dev. Biol.*, **157**, 85–99.

Martin, F.H., Suggs, S.V., Langley, K.E., Lu, H.S., Ting, J., Okino, K.H., Morris, C.F., McNiece, I.K., Jacobsen, F.W., Mendiaz, E.A. *et al.* (1990) Primary structure and functional expression of rat and human stem cell factor DNAs. *Cell*, **63**, 203–211.

Martini, E., Geraedts, J.P., Liebaers, I., Land, J.A., Capitanio, G.L., Ramaekers, F.C. and Hopman, A.H. (1996) Constitution of semen samples from XYY and XXY males as analysed by in- situ hybridization. *Hum. Reprod.*, **11**, 1638–1643.

Matsui, Y., Zsebo, K.M. and Hogan, B.L. (1990) Embryonic expression of a haematopoietic growth factor encoded by the Sl locus and the ligand for c-kit. *Nature*, **347**, 667–669.

Mauduit, C. (1999) Stem cell factor/c-kit system in spermatogenesis. *Hum. Rep. Update*, **5**, 535–545.

Maya-Nunez, G., Zenteno, J.C., Ulloa-Aguirre, A., Kofman-Alfaro, S. and Mendez, J.P. (1998) A recurrent missense mutation in the KAL gene in patients with X-linked Kallmann's syndrome. *J. Clin. Endocrinol. Metab.*, **83**, 1650–1653.

Mazeyrat, S., Saut, N., Sargent, C.A., Grimmond, S., Longepied, G., Ehrmann, I.E., Ellis, P.S., Greenfield, A., Affara, N.A. and Mitchell, M.J. (1998) The mouse Y chromosome interval necessary for spermatogonial proliferation is gene dense with syntenic homology to the human AZFa region. *Hum. Mol. Genet.*, **7**, 1713–1724.

McCarrey (1993) Development of the germ cell. In Desjardins, E.L. (ed.) *Cell and Molecular Biology of the Testis.* Oxford University Press, New York, USA, pp. 58–89.

McElreavey, K., Krausz, C. and Bishop, C.E. (2000) The human Y chromosome and male infertility. In McElreavey, K. (ed.) *The Genetic Basis of Male Infertility.* Springer-Verlag, New York, USA, pp. 211–232.

Meehan, T., Loveland, K.L., de Kretser, D., Cory, S. and Print, C.G. (2001) Developmental regulation of the bcl-2 family during spermatogenesis: insights into the sterility of bcl-w-/- male mice. *Cell. Death Differ.*, **8**, 225–233.

Mendis-Handagama, S.M., Risbridger, G.P. and de Kretser, D.M. (1987) Morphometric analysis of the components of the neonatal and the adult rat testis interstitium. *Int. J. Androl.*, **10**, 525–534.

Mendoza, T.A. (1992) Defective function of a nongenomic progesterone receptor as a sole sperm anomaly in infertile patients. *Fertil. Steril.*, **58**, 793–797.

Mickle, J., Milunsky, A., Amos, J.A. and Oates, R.D. (1995) Congenital unilateral absence of the vas deferens: a heterogeneous disorder with two distinct subpopulations based upon aetiology and mutational status of the cystic fibrosis gene. *Hum. Reprod.*, **10**, 1728–1735.

Oltvai, Z.N., Milliman, C.L. and Korsmeyer, S.J. (1993) Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell*, **74**, 609–619.

Orr-Urtreger, A., Avivi, A., Zimmer, Y., Givol, D., Yarden, Y. and Lonai, P. (1990) Developmental expression of c-kit, a proto-oncogene encoded by the W locus. *Development*, **109**, 911–923.

Orth, J.M. (1982) Proliferation of Sertoli cells in fetal and postnatal rats: a quantitative autoradiographic study. *Anat. Rec.*, **203**, 485–492.

Packer, A.I., Besmer, P. and Bachvarova, R.F. (1995) Kit ligand mediates survival of type A spermatogonia and dividing spermatocytes in postnatal mouse testes. *Mol. Reprod. Dev.*, **42**, 303–310.

Palombi, F., Farini, D., Salanova, M., de Grossi, S. and Stefanini, M. (1992) Development and cytodifferentiation of peritubular myoid cells in the rat testis. *Anat. Rec.*, **233**, 32–40.

Patrizio, P., Silber, S., Ord, T., Balmaceda, J.P. and Asch, R.H. (1988) Two births after microsurgical sperm aspiration in congenital absence of vas deferens. *Lancet*, **ii**, 1364.

Patrizio, P., Asch, R.H., Handelin, B. and Silber, S.J. (1993) Aetiology of congenital absence of vas deferens: genetic study of three generations. *Hum. Reprod.*, **8**, 215–220.

Patrizio, P. and Leonard, D.G.B. (2000) Mutations of the cystic fibrosis gene and congenital absence of the vas deferens. In McElreavey, K. (ed.) *The Genetic Basis of Male Infertility.* Springer-Verlag, Berlin, Germany, pp. 175–186.

Print, C.G. and Loveland, K.L. (2000) Germ cell suicide: new insights into apoptosis during spermatogenesis. *Bioessays*, **22**, 423–430.

Print, C.G., Loveland, K.L., Gibson, L., Meehan, T., Stylianou, A., Wreford, N., de Kretser, D., Metcalf, D., Kontgen, F., Adams, J.M., and Cory, S. (1998) Apoptosis regulator bcl-w is essential for spermatogenesis but appears otherwise redundant. *Proc. Natl Acad. Sci. USA*, **95**, 12424–12431.

Prosser, J., Inglis, J.D., Condie, A., Ma, K., Kerr, S., Thakrar, R., Taylor, K., Cameron, J.M. and Cooke, H.J. (1996) Degeneracy in human multi-copy RBM (YRRM), a candidate spermatogenesis gene. *Mamm. Genome*, **7**, 835–842.

Pryor, J.L., Kent-First, M., Muallem, A., Van Bergen, A.H., Nolten, W.E., Meisner, L. and Roberts, K.P. (1997) Microdeletions in the Y chromosome of infertile men. *N. Engl. J. Med.*, **336**, 534–539.

Reijo, R., Lee, T.Y., Salo, P., Alagappan, R., Brown, L.G., Rosenberg, M., Rozen, S., Jaffe, T., Straus, D., Hovatta, O. *et al.* (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat. Genet.*, **10**, 383–393.

Reijo, R., Alagappan, R.K., Patrizio, P. and Page, D.C. (1996) Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet*, **347**, 1290–1293.

Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.L. *et al.* (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science*, **245**, 1066–1073.

Rodriguez, I., Ody, C., Araki, K., Garcia, I. and Vassalli, P. (1997) An early and massive wave of germ cell apoptosis is required for the development of functional spermatogenesis. *EMBO J.*, **16**, 2262–2270.

Rommens, J.M., Iannuzzi, M.C., Kerem, B., Drumm, M.L., Melmer, G., Dean, M., Rozmahel, R. Cole, J.L., Kennedy, D., Hidaka, N. *et al.* (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science*, **245**, 1059–1065.

Ruggiu, M., Speed, R., Taggart, M., McKay, S.J., Kilanowski, F., Saunders, P., Dorin, J. and Cooke, H.J. (1997) The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. *Nature*, **389**, 73–77.

Russell, L.D., Ettlin, R.A., Hikim, A.P.S. and Clegg, E.D. (1990) *Histological and Histopathological Evaluation of the Testes.* Cache River Press, Florida, USA.

Sargent, C.A., Boucher, C.A., Kirsch, S., Brown, G., Weiss, B., Trundley, A., Burgoyne, P., Saut, N., Durand, C., Levy, N. *et al.* (1999) The critical region of overlap defining the AZFa male infertility interval of proximal Yq contains three transcribed sequences. *J. Med. Genet.*, **36**, 670–677.

Saxena, R., Brown, L.G., Hawkins, T., Alagappan, R.K., Skaletsky, H., Reeve, M.P., Reijo, R., Rozen, S., Dinulos, M.B., Disteche, C.M. and Page, D.C. (1996) The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. *Nat. Genet.*, **14**, 292–299.

Shan, Z., Hirschmann, P., Seebacher, T., Edelmann, A., Jauch, A., Morell, J., Urbitsch, P. and Vogt, P.H. (1996) A SPGY copy homologous to the mouse gene Dazla and the *Drosophila* gene boule is autosomal and expressed only in the human male gonad. *Hum. Mol. Genet.*, **5**, 2005–2011.

Silber, S.J., Patrizio, P. and Asch, R.H. (1990) Quantitative evaluation of spermatogenesis by testicular histology in men with congenital absence of the vas deferens undergoing epididymal sperm aspiration. *Hum. Reprod.*, **5**, 89–93.

Silber, S.J., Nagy, Z., Liu, J., Tournaye, H., Lissens, W., Ferec, C., Liebaers, I., Devroey, P. and Van Steirteghem, A.C. (1995) The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. *Hum. Reprod.*, **10**, 2031–2043.

Slee, R., Grimes, B., Speed, R., Taggart, M., Maguire, S. and Ross, A. (1999) A human DAZ transgene confers partial rescue of the mouse dazl null phenotype. *Proc. Natl Acad. Sci. USA*, **96**, 8040–8045.

Soulard, M., BValle, V.D., Siomi, M., Pinol-Roma, S., Codogno, P., Bauvy, C. and Belline, M. (1993) HnRNPG: sequence and characterisation of a glycosylated RNA-binding protein. *Nucleic Acids Res.*, **21**, 4210–4217.

Spratt, D.I., Carr, D.B., Merriam, G.R., Scully, R.E., Rao, P.N. and Crowley, W.F. Jr (1987) The spectrum of abnormal patterns of gonadotropin-releasing hormone secretion in men with idiopathic hypogonadotropic hypogonadism: clinical and laboratory correlations. *J. Clin. Endocrinol. Metab.*, **64**, 283–291.

Sun, C., Skaletsky, H., Birren, B., Devon, K., Tang, Z., Silber, S., Oates, R. and Page, D.C.. (1999) An azoospermic man with a de novo point mutation in the Y-chromosomal gene USP9Y. *Nat. Genet.*, **23**, 429–432.

Tiepolo, L. and Zuffardi, O. (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum. Genet.*, **34**, 119–124.

Tietze, C. (1956) Statistical contribution to the study of human fertility. *Fertil. Steril.*, **7**, 88–95.

Tietze, C. (1968) Fertility after discontinuation of intrauterine and oral contraception. *Int. J. Fertil.*, **13**, 385–389.

Tournaye, H., Devroey, P., Liu, J., Nagy, Z., Lissens, W. and Van Steirteghem, A. (1994) Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil. Steril.*, **61**, 1045–1051.

Tut, T.G., Ghadessy, F.J., Trifiro, M.A., Pinsky, L. and Yong, E.L. (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production and male infertility. *J. Clin. Endocrinol. Metab.*, **82**, 3777–3782.

Vandenbark, G.R., deCastro, C.M., Taylor, H., Dew-Knight, S. and Kaufman, R.E. (1992) Cloning and structural analysis of the human c-kit gene. *Oncogene*, **7**, 1259–1266.

Vergnaud, G., Page, D.C., Simmler, M.C., Brown, L., Rouyer, F., Noel, B., Botstein, D., de la Chapelle, A. and Weissenbach, J. (1986) A deletion map of the human Y chromosome based on DNA hybridization. *Am. J. Hum. Genet.*, **38**, 109–124.

Vogt, P., Chandley, A.C., Hargreave, T.B., Keil, R., Ma, K. and Sharkey, A. (1992) Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of AZF, a human spermatogenesis gene. *Hum. Genet.*, **89**, 491–496.

Vogt, P.H., Edelmann, A., Kirsch, S., Henegariu, O., Hirschmann, P., Kiesewetter, F., Kohn, F.M., Schill, W.B., Farah, S., Ramos, C. *et al.* (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum. Mol. Genet.*, **5**, 933–943.

Vollrath, D., Foote, S., Hilton, A., Brown, L.G., Beer-Romero, P., Bogan, J.S. and Page, D.C. (1992) The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science*, **258**, 52–59.

Waldstreicher, J., Seminara, S.B., Jameson, J.L., Geyer, A., Nachtigall, L.B., Boepple, P.A., Holmes, L.B. and Crowley, W.F. Jr (1996) The genetic and clinical heterogeneity of gonadotropin-releasing hormone deficiency in the human. *J. Clin. Endocrinol. Metab.*, **81**, 4388–4395.

Wang, R.A., Nakane, P.K. and Koji, T. (1998) Autonomous cell death of mouse male germ cells during fetal and postnatal period. *Biol. Reprod.*, **58**, 1250–1256.

World Health Organization (1987) Towards more objectivity in diagnosis and management of male infertility. *Int J. Androl*, **7**, 1–53.

World Health Organization (1992) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.* Cambridge University Press, Cambridge, UK.

Yan, W., Suominen, J. and Toppari, J. (2000) Stem cell factor protects germ cells from apoptosis *in vitro. J. Cell Sci.*, **113**, 161–168.

Yarden, Y., Kuang, W.J., Yang-Feng, T. Coussens, L., Munemitsu, S., Dull, T.J., Chen, E., Schlessinger, J., Francke, U. and Ullrich, A. (1987) Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J.*, **6**, 3341–3351.

Yen, P.H. (1998) A long-range restriction map of deletion interval 6 of the human Y chromosome: a region frequently deleted in azoospermic males. *Genomics*, **54**, 5–12.

Yen, P.H., Chai, N.N. and Salido, E.C. (1997) The human DAZ genes, a putative male infertility factor on the Y chromosome, are highly polymorphic in the DAZ repeat regions. *Mamm. Genome*, **8**, 756–759.

Yoshinaga, K., Nishikawa, S., Ogawa, M., Hayashi, S., Kunisada, T. and Fujimoto, T. (1991) Role of c-kit in mouse spermatogenesis: identification of spermatogonia as a specific site of c-kit expression and function. *Development*, **113**, 689–699.

Zsebo, K.M., Williams, D.A., Geissler, E.N., Broudy, V.C., Martin, F.H., Atkins, H.L., Hsu, R.Y., Birkett, N.C., Okino, K.H., Murdock, D.C. *et al.* (1990) Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell*, **63**, 213–224.

*Submitted on October 4, 2001; accepted on January 29, 2002*