Natural history of seminiferous tubule degeneration in Klinefelter syndrome

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Klinefelter syndrome (47,XXY) is characterized by small, firm testis, gynaecomastia, azoospermia and hypergonadotropic hypogonadism. Degeneration of the seminiferous tubules in 47,XXY males is a well-described phenomenon. It begins in the fetus, progresses through infancy and accelerates dramatically at the time of puberty with complete hyalinization of the seminiferous tubules, although a few tubules with spermatogenesis may be present in adult life. Activation of the pituitary-gonadal axis at 3 months of age is seen in Klinefelter boys similar to healthy boys. However, the level of testosterone in Klinefelter boys is significantly lower than in controls. After this ‘minipuberty’, the hormone levels decline to normal prepubertal levels until puberty. In puberty, an initial rise in testosterone, inhibin B, LH and FSH occurs in Klinefelter boys. However, the rise in testosterone levels off and ends at a low-normal level in young adults. Likewise, serum concentration of inhibin B exhibits a dramatic decline to a low, often undetectable level, concomitantly with a rise in FSH, reflecting the degeneration of the seminiferous tubules. Many hypotheses about the underlying mechanism of the depletion of the germ cells in Klinefelter males have been reported and include insufficient supranumerary X-chromosome inactivation, Leydig cell insufficiency and disturbed regulation of apoptosis of Sertoli and Leydig cells. However, at present, the exact mechanism remains unclear. In this article, we summarize current knowledge on the development of the classical endocrinological and histological features of 47,XXY males from fetus to adulthood and review the literature concerning the degeneration of the seminiferous tubules in this syndrome.

Key words: azoospermia/infertility/Klinefelter syndrome/Sertoli cells/testes

Introduction

Klinefelter syndrome was first described as a clinical entity by Harry F. Klinefelter in 1942 (Klinefelter, 1942), and the disorder was subsequently found to be caused by the presence of an extra X chromosome (Jacobs and Strong, 1959). Today, it is known that approximately 80% of the cases are because of the numerical chromosome aberration 47,XXY; the remaining 20% have higher-grade chromosome aneuploidies (e.g. 48,XXXY) or mosaicsisms (Foeresta et al., 1998). The syndrome is the most common sex-chromosome abnormality occurring in approximately 1 in 600 newborn males (Bojesen et al., 2003), and it is the most frequent genetic cause of infertility occurring in 11% of azoospermic men (Van Assche et al., 1996; Foeresta et al., 1999). Phenotypically, the Klinefelter male is characterized by small firm testes, gynaecomastia, eunuchoid body proportions, azoospermia, high levels of gonadotrophins (FSH and LH) and low normal levels of testosterone (Paulsen et al., 1968). It is known that the phenotype of Klinefelter males progressively deviates from normal with the increasing number of extra X chromosomes present, whereas the Klinefelter males with mosaicism most often are less severely affected (Lanfranco et al., 2004). The presence of a few cells with normal karyotypes (low-grade mosaicism) may be related to a preservation of some germ cells (Lenz et al., 2005), hereby influencing the fertility potential.

Klinefelter syndrome may be associated with an increased risk of certain systemic diseases including malignancies, autoimmune diseases (e.g. diabetes mellitus, hypothyroidism and rheumatic diseases), osteoporosis and venous thromboembolism (Campbell and Price, 1981; Kubler et al., 1992; Oktenli et al., 2002), although controversy exists. In a nationwide Danish registry study of 781 Klinefelter males, the syndrome was found to be associated with an increased mortality risk of 40% and a reduction in median survival of 2.1 years compared with controls (Bojesen et al.,...
2004). The increased mortality was mainly because of infectious, neurological, circulatory, pulmonary and urinary tract diseases (Bojesen et al., 2004).

Because of the substantial variation in clinical presentation and the relatively discrete symptoms, especially before puberty, most of the patients are never diagnosed. Less than 10% of all subjects with Klinefelter syndrome are diagnosed before puberty, and only approximately one fourth of adult males with the syndrome are diagnosed (Bojesen et al., 2003).

The extra X chromosome in Klinefelter syndrome causes infertility because of the degeneration of the germ cells. When and why the germ cells degenerate are important but not yet fully answered questions. To date, various studies have tried to address this subject. However, the results have been difficult to interpret owing to problems of late diagnosis and tissue sampling resulting in small sample sizes. Thus, most of the available data on Klinefelter syndrome are based on studies of less than 20 patients. The increasing use of amniocentesis, however, has made it possible to examine fetuses with the Klinefelter karyotype and to follow boys with Klinefelter syndrome from birth onwards and, thereby, to describe in more detail the testicular degeneration process in Klinefelter males. Some reports suggest that the degeneration of germ cells starts in early infancy, leading to the absence of or to a significantly reduced number of germ cells even before puberty (Mikamo et al., 1968; Murken et al., 1974; Ratcliffe, 1982; Coerdt et al., 1985; Muller et al., 1999). Complete absence of germ cells is, however, not always the rule. Even azoospermic patients may have focal spermatogenesis in the testis and may therefore benefit from assisted reproductive techniques to father a child (Denschlag et al., 2004).

The aim of this article is to review the existing knowledge on testicular development in subjects with Klinefelter syndrome with particular emphasis on the ontogeny of histological and hormonal changes associated with germ cell demise.

**Histopathology of Klinefelter testis during development**

The degeneration of the germ cells in Klinefelter syndrome, which is a well-documented phenomenon may be because of a primary effect of the extra X chromosome on the development and function of the germ cells or adverse influence on the supporting somatic cells including the Leydig and Sertoli cells.

Testosterone, produced by Leydig cells, plays an indispensable role in spermatogenesis (Rey, 2003). High intratesticular, rather than circulating levels of testosterone, and an adequate expression of androgen receptors in Sertoli cells are necessary for the onset of puberty (Rey, 2003).

The Sertoli cells, possibly together with the adjacent basement membrane, create a particular microenvironment which controls the renewal and differentiation of the germ cells by providing nutrition, adhesion and several transport functions (Kerr, 1992; Print and Loveland, 2000; Spradling et al., 2001).

In the following section, available data on the age-specific changes in histology (Figure 1) and hormone levels in individuals with Klinefelter syndrome are described.

**The fetal period**

From the studies of fetuses aborted at a gestational age of 18–22 weeks, we know that the degenerative process has already started at this early stage (Murken et al., 1974; Autio-Harmainen et al., 1980; Coerdt et al., 1985). Thus, a significantly reduced number of germ cells were seen in studies of testicular biopsies from 47,XXY mid-term fetuses, whereas the density and number of testicular tubules and mesenchymal structures appeared normal (Coerdt et al., 1985).

The number of germ cells was even further reduced in a fetus with undisceded testes aborted at 20 weeks of gestation (Murken et al., 1974) and in an infant with inguinal hernia, who underwent surgery at 4 weeks of age (Ratliffe, 1982). Leydig cells have appeared morphologically normal in all but one of the studies (Murken et al., 1974). However, the testicular biopsy in the latter study was taken from a Klinefelter fetus with testes not yet descended, and similar histological changes are seen in cryptorchid males with a normal karyotype (Nistal, 1982; Regadera et al., 1999).

**Neonatal period and ‘minipuberty’**

There may be some impairment of the Leydig cell function at birth, even though this has not been confirmed histologically (Sorensen et al., 1981; Ratcliffe, 1982; Lahlou et al., 2004). Sorensen et al. (1981) measured cord-blood testosterone in two 47,XXY infants and one with mosaicism (46,XX/47,XXY) and found significantly lower levels compared with three control infants. This study was only based on three patients and three controls. However, when the testosterone levels of the three Klinefelter

![Figure 1. Testicular histology in Klinefelter syndrome during development. A. fetal testis; B. a 4-year-old boy with a relatively large number of germ cells; C–E, three prepubertal 10- to 12-year-old patients with variable numbers of remaining germ cells, note a few focally grouped tubules with germ cells; visible on the right side of the figure in D, and no germ cells remaining in E, and F, a 14-year-old without germ cells present, note degeneration of tubules and Leydig-cell nodules.](image-url)
infants were compared with the levels of testosterone in a larger cohort of control infants, there was no difference (Ratcliffe, 1982).

The pituitary–gonadal axis in healthy boys is strongly activated after birth—which is manifested by pubertal or even adult levels of serum FSH, LH, testosterone and inhibin B at the age of 3 months—the so called ‘minipuberty’ (Andersson et al., 1998b). Hereafter, the hormone levels decline to normal prepubertal levels until the pubertal reactivation of the axis occurs. The occurrence of the ‘minipuberty’ represents a window, where it is possible to study the function of the pituitary–gonadal axis by measuring the spontaneous, basal hormone levels (Main et al., 2002). Lahlou et al. published a study in 2004, in which they compared the gonadotropin and reproductive hormone levels in 215 healthy infants with the hormone levels of 18 infants with prenatally diagnosed nonmosaic Klinefelter syndrome. The infants with Klinefelter had a testosterone peak during the first months of life similar to that of control infants, but the testosterone levels were significantly lower from birth to 8 months in the infants with Klinefelter syndrome, suggesting an impaired Leydig cell function already at this early age. By contrast, LH, FSH, inhibin B and Anti-Mullerian Hormone (AMH) levels were normal (Lahlou et al., 2004). In accordance with the normal histology of the Sertoli cells as reported in earlier studies, this finding suggests that the Sertoli cells are qualitatively and quantitatively normal in 47,XXX children in infancy (Mikamo et al., 1968; Ratcliffe, 1982; Lahlou et al., 2004).

The childhood and adolescence

Several studies of prepubertal Klinefelter boys have revealed a similar testicular histology as already described in the Klinefelter fetus. All studies have shown preservation of seminiferous tubules with a reduced number of germ cells, whereas the Sertoli cells and Leydig cells have appeared (when described) normal and of juvenile type (Ferguson-Smith, 1959; Mikamo et al., 1968; Muller et al., 1995; Wikstrom et al., 2004). Wikstrom et al. (2004) published a study of 14 nonmosaic Klinefelter boys aged 10–14 years. Importantly, none of the boys enrolled were cryptorchid or undergoing androgen substitution. The biopsies of pre- and peripubertal boys showed germ cells, but the number of spermatagonia present was markedly reduced and no meiotically dividing germ cells or postmeiotic spermatids appeared in any of the biopsies (Wikstrom et al., 2004). The presence of germ cells in peripubertal Klinefelter boys contrasts with the results of Muller et al. (1995), who found no germ cells in biopsies from Klinefelter boys aged 2 years or more. However, in the latter study, all boys were cryptorchid, a condition, which has also a detrimental effect on the seminiferous epithelium.

The prepubertal 47,XXX boys were characterized by normal levels of testosterone, FSH, LH and inhibin B until the onset of puberty (Topper et al., 1982; Salbenblatt et al., 1985; Christiansen et al., 2003; Wikstrom et al., 2004). Major histological changes in the testes coincided with the pubertal activation of the pituitary–gonadal axis. As the Klinefelter boys entered puberty, their testis initially grew up to a volume of 6 ml. However, as serum-testosterone levels increased, the depletion of germ cells, the hyalinization of the tubules, the degeneration of the Sertoli cells and the hyperplasia of the Leydig cells accelerated (Wikstrom et al., 2004). This was associated with a decrease in the testis volumes to a prepubertal size of 2–4 ml (Ratcliffe et al., 1986; Robinson et al., 1986). The degeneration process was accompanied by a relative Leydig-cell insufficiency reflected by the impaired serum testosterone levels and increasing LH levels. The initial adolescent rise in testosterone was relatively normal, but from the age of about 14 years serum concentrations of testosterone levelled off and remained in the low-normal range through puberty (Topper et al., 1982; Salbenblatt et al., 1985; Wikstrom et al., 2004). It remains uncertain whether the rise in serum or intratesticular testosterone concentrations in puberty is associated with the accelerated destruction of the seminiferous tubules in puberty. The level of inhibin B is known to increase before puberty, but as the testosterone level increased the inhibin B level was very rapidly suppressed with a concomitant rise in serum FSH levels (Christiansen et al., 2003; Wikstrom et al., 2004). Thus, inhibin B was most often undetectable at the end of puberty in Klinefelter patients (Christiansen et al., 2003; Wikstrom et al., 2004). In pre- and peripubertal boys, the production of inhibin B is known to be regulated by Sertoli cells. After the onset of puberty and in adult males, however, the inhibin B production becomes germ-cell dependent, and serum inhibin B can thus no longer serve as an exclusive marker of Sertoli-cell function (Andersson et al., 1998a) (Figure 2).

Adult life

As described by Klinefelter in 1942, the histology of the testes is characterized by extensive fibrosis and hyalinization of the seminiferous tubules, and hyperplasia of interstitium in the adult

Figure 2. Longitudinal assessment of serum FSH (top panel) and inhibin B (lower panel) in 36 untreated children and adolescents with Klinefelter syndrome (Part of data previously published by Christiansen et al., 2003). The solid lines represent mean ± 2 SD for healthy boys (Andersson et al., 1997).
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patient (Klinefelter, 1942). Many studies have since then evidenced the patchy nature of the testicular histology with more- and less-affected areas (Heller, 1945; Steinberger, 1965; Skakkebaek, 1969; Skakkebaek et al., 1969; Nistal, 1982; Forresta et al., 1999; Wikstrom et al., 2004). Forrestra and colleagues studied ten 47,XXY males aged 28–37 years and found Sertoli-cell-only pattern in eight of ten biopsies, whereas the remaining two showed Sertoli cells and few spermatogenic cells (Forresta et al., 1999). In 1969, Skakkebaek described two types of tubules in relation to Sertoli-cell morphology containing either small immature Sertoli cells (chromatin positive) or larger and more differentiated Sertoli cells (chromatin negative) (Figure 3). Later, these immature Sertoli cells were studied further and were found to have an impaired physiological activity resulting in a compromised protein and steroid hormone synthesis (Nistal, 1982). Thus, the adult Klinefelter patients are characterized by hypergonadotropic hypogonadism as evidenced by low to low-normal levels of testosterone, high FSH and LH levels, and undetectable levels of serum inhibin B in most of the patients (Salbenblatt et al., 1985; Anawalt et al., 1996; Forresta et al., 1999; Christiansen et al., 2003; Lahhou et al., 2004; Lanfranco et al., 2004; Wikstrom et al., 2004).

Fertility in subjects with Klinefelter syndrome

The Klinefelter subjects are traditionally described as infertile because of a complete absence of germ cells. Although semen analysis most often reveals azoospermia, some Klinefelter men may have single-residual foci with spermatogenesis (Heller, 1945; Ferguson-Smith, 1959; Steinberger, 1965; Skakkebaek, 1969; Skakkebaek et al., 1969; Froland and Skakkebaek, 1971; Forresta et al., 1999). It is believed that some spermatogenesis in Klinefelter subjects is capable of completing the spermatogenic process leading to the formation of mature spermatozoa (Froland et al., 1999; Bergere et al., 2002) (Figure 3F). However, natural conception rarely occurs for Klinefelter couples, and most often the only hope for biological paternity is testicular sperm extraction (TESE) combined with ICSI. The initial success rate of TESE in adult 47,XXY males in a small series was reported to be 40–50% (Lanfranco et al., 2004). The fact that the germ-cell degeneration accelerates dramatically at the onset of puberty makes it tempting to retrieve germ cells at an earlier age for cryopreservation and future utilization (Damani et al., 2001; Lin et al., 2004). However, Wikstrom et al. (2004) found that only 50% of the Klinefelter boys had germ cells in their testes indicating a severely impaired fertility potential even in the peripubertal period.

The levels of FSH, inhibin B and the inhibin B/FSH ratio are known predictive factors for fertility in males with normal karyotype (Andersson et al., 1997). In the Klinefelter male, the only predictive factor for a successful germ cell recovery seems to be the testicular histopathology (Westlander et al., 2001), but even when no sperm is found in a biopsy, TESE has been successful (Vernaeve et al., 2004). The predictive value of testicular volume, testosterone levels and response to HCG test for successful TESE was shown in one study (Madgar et al., 2002), but not in others (Westlander et al., 2001; Vernaeve et al., 2004). FSH, LH and inhibin B levels were not predictive for successful TESE. Likewise, testicular ultrasonography, intratesticular blood-flow resistance, degree of virilization or extensive chromosome analyses did not seem to predict the outcome of TESE (Westlander et al., 2001; Vernaeve et al., 2004b). In fact, even patients with inhibin B below the detection limit underwent successful TESE (Westlander et al., 2003). Vernaeve et al. (2002, 2004a) have confirmed that inhibin B is an established marker of spermatogenesis, but not a predictive factor for the outcome of TESE in males with nonobstructive azoospermia. To date, more than 40 healthy children of Klinefelter fathers have been born following the use of ICSI (Denschlag et al., 2004). A triplet gestation, where one 47,XXY fetus was reduced, has been reported (Ron-El et al., 2000). Because of the hypothetical risk of producing a sex chromosomal abnormality in the offspring, most investigators recommend professional genetic counselling and standard prenatal diagnostic techniques (Denschlag et al., 2004).

Other features of the Klinefelter phenotype

There is a wide variation in the Klinefelter phenotype. Skewed X-chromosome inactivation, frequently seen in females, defined as greater than 80% preferential inactivation of one of the two X chromosomes, occurs also in Klinefelter syndrome (Iitsuka et al., 2001). It is possible that preferential inactivation of genes may influence the Klinefelter phenotype, but this remains to be elucidated.
One gene that is of physiological importance in the testis is the androgen-receptor gene (AR, mapped to Xq11.2-12). The AR gene contains a polymorphic stretch of CAG repeats in exon 1. The length of this stretch is inversely related to the receptor’s basal and ligand-induced activity in vitro and may influence physiological response to androgens (Zitzmann and Nieschlag, 2003). In the Klinefelter males, one of the two AR alleles is inactivated (Iitsuka et al., 2001; Suzuki et al., 2001; Zitzmann et al., 2004). Zitzmann et al. (2004) studied the CAG polymorphism in 77 Klinefelter males and found evidence for a preferential inactivation of the shorter allele. Conversely Suzuki et al. (2001) studied 13 47,XXY males and reported preferential inactivation of the longer allele. Furthermore, Zitzmann et al. (2004) demonstrated that Klinefelter males with a longer CAG repeat tend to be more severely affected than those with a shorter CAG stretch in the AR. This association was found in relation to socioeducational status, growth pattern, bone density and occurrence of gynecomastia. The effect of testosterone treatment was also correlated to the length of the CAG repeat in the AR. Males with short repeats responded to testosterone substitution with a more pronounced suppression of LH and larger increment of testosterone levels than males with longer CAG repeats in the AR.

The variation in the CAG length can, at least partially, explain the variation in the Klinefelter phenotype, as this polymorphism was linked to variability in some androgen-dependent functions in normal healthy 46,XY males (Zitzmann and Nieschlag, 2003). That many Klinefelter males present the classical hypogonadal phenotype even though they have testosterone levels in the low-normal range might reflect some degree of androgen resistance as suggested by the high-normal LH levels as well as from the demonstrated associations between number of the CAG repeats in the AR and phenotypical characteristics in Klinefelter males. In line with these findings, Zinn et al. (in press) found an inverse relation between the number of CAG repeats and penile length in 35 Klinefelter boys and men, but did not find any associations with height, BMI, head circumference, testicular volume or presence of gynecomastia.

Lessons from animal models

The development of the XXY mouse in 1991 provided a tool to investigate the development of germ cells (Hunt and Eicher, 1991). It has initially been suggested that the reduction in germ cells was the result of a difference in the number of germ cells colonizing the genital ridges. However, Hunt et al. (1998) counted the primordial germ cells in the genital ridges of the XXY mice at different developmental stages of the gonads and found no significant difference in comparison with XY controls. This suggested that normal numbers of primordial germ cells arrived at the genital ridges of the XXY embryo, and the impairment of the mitotic proliferation became only evident after the differentiation of the testis had begun (Hunt et al., 1998). In 1998, Hunt et al. found a reduction in the number of germ cells per tubule cross section at all stages from the prenatal period onwards in XXY mice. Furthermore, they did not find any signs of post-natal mitotic proliferation but rather a progressive decline in the number of germ cells during early post-natal period until 12 days post-partum, where they could hardly recognize any remaining germ cells (Hunt et al., 1998).

In the same manner, Lue et al. (2001) found that germ cells began to degenerate in the XXY mice at 7 days of age with a progressive loss resulting in a total absence of germ cells in the adult animals. Hypertrophy and hyperplasia of the Leydig cells and changes in the Sertoli cells indicating cellular inactivity were furthermore observed.

Another recent study of XXY mice confirmed the abovementioned observations and added new knowledge to the mechanisms of Sertoli-cell degeneration and thereby the mechanism behind germ-cell loss. The investigators found that by the age of 20 days, there was a sporadic loss of AR expression in some of the Sertoli cells of the XXY mice resulting in a complete loss of AR expression in the adult XXY mice. Since Sertoli cells have a supporting influence on spermatogenesis, the resulting dysfunction of the Sertoli cells might be a major factor responsible for the loss of germ cells in early age (Lue et al., 2005). Lue et al. (2005) furthermore found differences in the intracellular localization of the AR in the Leydig cells of XY and XXY mice. In XXY mice, the AR was only found in the cytoplasm, whereas the AR was present in the nuclei in XY mice representing a possible reason for the impaired Leydig-cell function in adult XXY mice.

Trying to elucidate whether the XXY somatic environment of the testis influenced the degeneration of germ cells, Hunt et al. (1998) studied the proliferative potential of the XXY-mice germ cells in vitro and found no significant changes. This was indicative of a dysfunction in the communication between the soma and germ cells that could be because of a failure in the proliferative signals from the soma or the ability of the germ cells to respond. Further investigations revealed no apparent abnormalities, neither in the developing Sertoli cells, nor in the production of testosterone in Leydig cells (Hunt and Eicher, 1991).

These studies of animal models helped to describe the germ-cell demise in detail but did not shed much light on the underlying mechanisms.

What are the mechanisms of germ-cell depletion?

One of the fundamental questions is whether the abnormal karyotype affects primarily germ cells or affects primarily somatic cells in the testis, in particular Sertoli cells, which are thought to be main mediators of signals from the outside to the germ-cell compartment. Leydig cells may also be affected, and it is therefore probable that the testicular phenotype is a result of impaired function and interaction of several cell types.

The acceleration of germ-cell demise occurs at the onset of puberty; when in the normal testis, the activation of reproductive hormones triggers the process of gamete production, which requires a switch from mitosis to meiosis. That led to one of earlier hypotheses that aneuploid germ cells cannot efficiently align during meiosis, and the unsynapsed chromosomes would disturb the meiotic checkpoint and trigger apoptosis at the pachyten phase of meiosis. Miklos, 1974; Burgoyne, 1993). If that hypothesis was true, the predominant histological picture would have been that of the maturation arrest at the level of spermatocyte, which is sometimes seen in patients with large deletions in the Y-chromosome’s male-specific region (known also as the azoospermia factor region, AZF). On the other hand, numerous reports suggested that aneuploid germ cells sometimes can slip through the meiotic checkpoint and mature to spermatozoa (Skakkebaek et al., 1969;
Chevret et al., 1996). Some scientists demonstrated by fluorescence in-situ hybridization (FISH) the presence of hyperhaploid (24,XX or 24,XY) spermatids in the vicinity of 47,XXY spermatogonia in presumably nonmosaic Klinefelter-syndrome patients (Yamamoto et al., 2002). Others dispute that (Egozcue et al., 2002) and claim that only 46,XY spermatogonia can complete meiosis, and their presence in Klinefelter patients is owing to the apparent low percentage mosaicism for sex chromosome aneuploidy (Blanco et al., 2001; Ekerhovd and Westlander, 2002; Lenz et al., 2005). The sex-chromosome disomy sometimes observed in the sperm of Klinefelter patients may then result from meiosis-II errors caused by impaired cellular microenvironment. The latter opinion is supported by the studies of the mouse XXY model, where only XY spermatogonia survived in the adults (Mroz et al., 1999). Discussion on this aspect is not yet over.

**Gene dosage and X-chromosome inactivation**

As described above in this review, the vast majority of the 47,XXY patients never experience meiosis, and their germ cells disappear at the mitotic stage of spermatogonia or during earlier differentiation. Therefore, a predominant hypothesis is that the altered dosage of some genes on X chromosome may affect the development and/or degeneration of the germ cells in males with 47,XXY (Spatz et al., 2004). It is well known that in females, one of the two X chromosomes is randomly inactivated in the somatic cells to obtain a gene dosage, which is equivalent to that in males. Although many genes escape inactivation, the inactivated X chromosome is microscopically visible as the Barr body (sex chromatin) in female cells (Barr, 1949). The inactivation of an extra X chromosome in human somatic cells is mediated primarily by a RNA product from the gene called X-inactivation-specific transcript (XIST) located on the long arm of the inactive X chromosome (Brown et al., 1999; Plath et al., 2002). Therefore, the expression of XIST is a marker of the presence of the second and any further extra X chromosomes in the somatic cell (Penny et al., 1996).

Somatic cells in the males with 47,XXY inactivate the supranumerary X chromosomes most probably in the same manner as the somatic cells in females. XIST is expressed in blood cells of Klinefelter men but not in the blood of healthy men with normal karyotype (Kleinheinz and Schulze, 1994). The Barr body was also found in Leydig and undifferentiated Sertoli cells in males with Klinefelter syndrome (Froland and Skakkebaek, 1971; Shamsuddin and Tang, 1980). Therefore, we assume that any increase in gene dosage in these cell types will only concern genes that escape inactivation.

As far as the possible role of X-mapped genes that escape inactivation is concerned, very little is known about their expression patterns. It is estimated that approximately 15% of X-linked genes escape inactivation to some degree, but there are many more that show cell-type-specific inactivation pattern (Carrel and Willard, 1999, 2005; Carrel et al., 1999). The genes that escape inactivation tend to cluster on the distal part of the short arm (Xp), whereas it is the long arm (Xq) that apparently contains genes, which primarily contribute to the Klinefelter phenotype. This is based on the reports of Klinefelter syndrome in patients with isochromosomes or other structural aberrations of Xq (Arps et al., 1996; Nemeth et al., 2002).

The situation in germ cells appears to be quite different and much more complex, because the ways of the X-chromosome inactivation in germ cells do not follow the pathways established in female somatic cells (Armstrong et al., 1997; Fernandez-Capetillo et al., 2003). Earlier studies assumed that the expression of XIST was synonymous with the X-chromosome inactivation, therefore it was concluded that the sole X chromosome in male germ cells was inactivated in the adult testis. This conclusion was mainly based on a study of fertile men who did express XIST in the testis, whereas males with Sertoli-cell-only syndrome did not (Salido et al., 1992). Thus, germ cells were the only cell type expressing XIST in the testis. A few years later, it became clear that the X-chromosome inactivation does not fully occur in adult spermatogonia, as it was shown that among the X-chromosome genes, a surprisingly large number was expressed in testicular germ cells (Wang et al., 2001). The recent sequencing of the X chromosome revealed that probably as many as 10% of protein-coding genes on the X chromosome may be testis specific and belonging to the so-called ‘cancer-testis antigens’ family (Ross et al., 2005). The name comes from the common transcription activation of these genes in various human cancers, e.g. melanomas, lung cancers (Scanlan et al., 2002). A high expression of some of these genes is also seen in fetal germ cells and testicular neoplasms, including preinvasive carcinoma in situ (CIS) and spermatocytic seminomas, but very rarely in nonseminomas, tumours which do not retain germ-cell-like phenotypic features (Aubry et al., 2001; Satie et al., 2002). Germ-cell cancer may serve to some extent as a model for the Klinefelter syndrome, because neoplastic germ cells in most cases display polyploidy and a significant amplification of the X-chromosome material (Peltomaki et al., 1989; Oosterhuis and Looijenga, 2005). The expression of some of the abovementioned ‘cancer-testis’ antigens and other X-linked genes was reported in germ-cell neoplasms and some derived cell lines, despite the activation of xist transcription (Looijenga et al., 1997; Kawakami et al., 2003; Almstrup et al., in press). Studies of the gene inactivation in male germ cells are still in their infancy, so the mechanisms have not been elucidated even in animal models. It is clear that in mice, XIST is not required for sex-chromosome inactivation in germ cells in mature testes (Turner et al., 2002), but the situation during very early development is not yet known. We expect that the recent progress in genomic analysis of the X chromosome as well as better understanding of epigenetic regulation of gene expression will soon shed some more light on this aspect of the biology of germ cells (Carrel and Willard, 2005; Ross et al., 2005).

**Klinefelter syndrome and germ-cell neoplasia**

It is noteworthy that Klinefelter patients carry an increased risk of extragonadal (mediastinal and intracranial) germ-cell cancer, and some other types of cancer, e.g. breast cancer. It follows, that the supranumerary X chromosome most probably provides a proliferative or survival advantage to germ cells during their migration to the gonadal ridges, but the opposite is true for the germ cells within the testis. The other important point is that the expression of some X-linked genes in male germ cells is developmentally regulated. For example, MAGE-A4 is not expressed in very early gonocytes (and most probably it is not active in primordial germ cells), whereas it is highly expressed in gonocytes from mid-gestation as well as in infantile and mature spermatogonia (Aubry et al., 2001). Therefore, it is possible that some testis-specific genes of the X chromosome become activated only when germ cells have completed their migration, or even later at the onset of meiosis.
Moreover, some of the X-chromosome genes are expressed in somatic cells in the testis, and we can speculate that increased expression of those that escape inactivation may affect the germ cells. Among the interesting genes to look at from this perspective, are, e.g., a gene encoding p120, a putative inhibin-binding protein (mapped to Xq24; Chong et al., 2000) or the angiotensin type-II receptor gene, AT2 (Xq21.3). The AT2 receptor may be of particular interest with regard to the quickly progressing demise of germ cells in Klinefelter males, because it mediates apoptosis in some cell types and is considered to be involved in the physiological atresia of ovarian follicles (Yamada et al., 1996; Kotani et al., 1999).

**Apoptosis**

Apoptosis is a mechanism responsible for the physiological regulation of germ-cell death during differentiation and maturation of normal human germ cells and could contribute to the excessive germ-cell demise in males with 47,XXY. Apoptosis is a prerequisite for continuous spermatogenesis (Print and Loveland, 2000), by selectively removing dysfunctional or damaged germ cells, and by limiting germ cell number (Grootegoed et al., 2000).

Gonadotrophins (FSH, LH) and testosterone are important regulators of germ-cell apoptosis (Sinha Hikim and Swerdloff, 1999; Print and Loveland, 2000). Their removal induces apoptosis, which occurs presumably through indirect effects, since hormone receptors are present on somatic cells (Print and Loveland, 2000). FSH is classically considered to be involved in the initiation of the pubertal spermatogenesis. It regulates DNA synthesis, proliferation and differentiation of spermatogonia and spermiogenesis (Rey, 2003). FSH inhibits male germ-cell apoptosis in cultured rat seminiferous tubules partially via stem-cell factor (SCF) produced by Sertoli cells and interacts with the c-kit receptor in the germ cells (Yan et al., 2000a,b,c). This mechanism may involve changes in the Bcl-2 family members, since in cultured rat seminiferous tubules, either FSH or Sertoli-cell-derived SCF can regulate antiapoptotic Bcl-w expression (Yan et al., 2000a,b,c). It could be hypothesized that the FSH receptor was malfunctioning or down-regulated in XXY Sertoli cells as FSH, which has pro-survival effects on germ cells, is considerably elevated in Klinefelter patients. This would be consistent with the observation that inhibin B is undetectable in Klinefelter males. The hypothetical malfunction of the FSH receptor might lower the SCF expression and thereby influence the ratio of pro- and anti-apoptotic factors early in development forcing the germ cells to undergo apoptosis.

In the human testis, testosterone is able to effectively inhibit in vitro-induced apoptosis of spermatocytes and spermatids (Erkkila et al., 1997). The anti-apoptotic action of testosterone may also be regulated by some of the testicular metabolites of testosterone, such as dihydrotestosterone and estrogens (Rey, 2003). Estrogens are potential regulators of male reproduction and germ-cell death. Low concentrations of 17β estradiol (10⁻⁹ and 10⁻¹⁰ mol/l) effectively inhibit male germ-cell apoptosis in the cultured human seminiferous tubules (Pentikainen et al., 2000). Estrogens can also cause alterations in circulating concentrations of gonadotrophins and testosterone and thus affect apoptosis in germ cells indirectly (O’Donnell et al., 2001; Pentikainen et al., 2003).

To our knowledge, no studies have directly addressed the possible relation between apoptosis and the degeneration of the seminiferous tubules in Klinefelter syndrome.

**Conclusions**

Severe degeneration of germ cells occurs in the testes of both XXY mice and humans. Descriptive studies of testicular histology clearly show that the disturbances of gonadal development of Klinefelter testes occurs very early in life and progress slowly through infancy with a dramatic acceleration in germ cell and Sertoli-cell degeneration at the onset of puberty. The histological changes found in infancy mainly concern the number of germ cells, whose reduction is highly significant. The endocrinological disturbances of Klinefelter males are likewise noticed already in the neonatal period. Puberty is however initiated with a normal rise in sex hormones but shortly after the level of testosterone levels off and ends at a low-normal level with elevated LH levels (relative hypogonadism). By contrast, inhibin B becomes extremely low or most often undetectable concomitantly with a markedly elevated FSH.

The underlying mechanisms of testicular degeneration are poorly understood. The different hypotheses concerning Leydig-cell insufficiency, impaired somatic environment of the testes, a dysfunctioning communication between the soma and the germ cells, incomplete X-chromosome inactivation as well as disturbed apoptotic activity of Leydig cells and Sertoli cells have been described. We believe that increased expression of genes located on the X chromosome that escape inactivation may play an important role. Existing evidence suggests a role for both germ-cell-specific genes and genes expressed in somatic testicular cells. The findings of the abundant expression of X-chromosome genes in the testis and the recent advances in understanding the genomic organization of the human X chromosome will undoubtedly contribute to a better understanding of the testicular phenotype in Klinefelter patients.

Most of the classical symptoms of Klinefelter syndrome can most probably be ascribed to the relative hypogonadism. It is therefore of utmost importance to detect this syndrome as early as possible to initiate androgen substitution and thereby hopefully prevent or ameliorate those features characterizing Klinefelter adults.

Most Klinefelter males are azoospermic, but some may have residual foci of spermatogenesis. Only few cases of spontaneous conception have been reported, therefore the Klinefelter patients with residual spermatogenesis may benefit from the ICSI treatment combined with the use of TESE. At present, however, we do not have any biochemical parameter for predicting the outcome of TESE. To date, birth of more than 40 healthy children of Klinefelter fathers following the use of ICSI have been reported (Denschlag et al., 2004).

Further collaborative studies regarding the efficacy of early androgen substitution as well as the possible fertility options for the Klinefelter patients are strongly needed.

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