Human Reproduction Update, Vol.12, No.3 pp. 303–323, 2006 doi:10.1093/humupd/dmk006
Advance Access publication March 15, 2006

Developmental model for the pathogenesis of testicular carcinoma *in situ*: genetic and environmental aspects

Ewa Rajpert-De Meyts

University Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

To whom correspondence should be addressed at: University Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Section GR-5064, 9 Blegdamsvej, DK-2100 Copenhagen, Denmark. Email: erm@rh.hosp.dk

Carcinoma *in situ* testis (CIS), also known as intratubular germ cell neoplasia (ITGCN), is a pre-invasive precursor of testicular germ cell tumours, the commonest cancer type of male adolescents and young adults. In this review, evidence supporting the hypothesis of developmental origin of testicular germ cell cancer is summarized, and the current concepts regarding aetiology and pathogenesis of this disease are critically discussed. Comparative studies of cell surface proteins (e.g. PLAP and KIT), some of the germ cell-specific markers (e.g. MAGEA4, VASA, TSPY and NY-ESO-1), supported by studies of regulatory elements of the cell cycle (e.g. p53, CHK2 and p19-INK4d) demonstrated a close similarity of CIS to primordial germ cells and gonocytes, consistent with the pre-meiotic origin of CIS. Recent gene expression profiling studies showed that CIS cells closely resemble embryonic stem cells (ESCs). The abundance of factors associated with pluripotency (NANOG and OCT-3/4) and undifferentiated state (AP-2γ) may explain the remarkable pluripotency of germ cell neoplasms, which are capable of differentiating to various somatic tissue components of teratomas. Impaired gonadal development resulting in the arrest of gonocyte differentiation and retention of its embryonic features, associated with an increasing genomic instability, is the most probable model for the pathogenesis of CIS. Genomic amplification of certain chromosomal regions, e.g. 12p, may facilitate survival of CIS and further invasive progression. Genetic studies, have so far not identified gene polymorphisms predisposing to the most common non-familial testicular cancer, but this research has only recently begun. Association of CIS with other disorders, such as congenital genital malformations and some forms of impaired spermatogenesis, all rising in incidence in a synchronous manner, led to the hypothesis that CIS might be a manifestation of testicular dysgenesis syndrome (TDS). The aetiology of TDS including testicular cancer remains to be elucidated, but epidemiological trends suggest a primary role for environmental factors, probably combined with genetic susceptibility.

Key words: carcinoma *in situ*/germ cell differentiation/embryonic stem cells/testicular cancer/testicular dysgenesis syndrome

Introduction

Testicular cancer is in most cases considered a disease of adults. Seeing a young man presenting with a testicular tumour or with symptoms of disseminated cancer disease, few clinicians would think that their patient's disease had been initiated long time before, during fetal development. However, evidence gathered over the last three decades and the newest findings support this hypothesis, as will be critically discussed in this review.

The early origin is only one of the unique features of testicular germ cell cancer. This neoplasm is unlike any other solid tissue cancer for a number of reasons, including unusual epidemiological and biological features. Epidemiological hallmarks include the peak incidence in a very young adult age, a markedly increasing incidence worldwide but with striking geographic and ethnic differences, and association with other reproductive conditions. Among particular biological features are the unusual histology characterized by extreme heterogeneity with components mimicking any tissue type of the body, including caricatural reflection of early embryos in teratomas, and the extreme sensitivity to irradiation and cytotoxic treatment.

One of the possible explanations for the unique biology of testicular germ cell cancer is that it is derived from germ cells, which are different from any other cells in the body because of their special function of exchanging and transferring hereditary information as gametes. Germ cells are the only cells that use two different types of cell division (mitosis and meiosis), and for that they require different regulation of cell cycle and DNA repair. The regulation of gene expression appears to be different as well, including waves of epigenetic activation and silencing, and a final selective chromosomal condensation during the process of spermiogenesis. In contrast to other cell types, germ cells retain

embryonic stem cell (ESC)-like features and pluripotency for a long time during development. For reasons not yet fully understood, perhaps because of this special hereditary role, germ cells and the reproductive system serving them appear to be exquisitely sensitive to changes in micro- and macro-environment. Research on these aspects has been energized in recent years after adverse epidemiological trends in male reproduction were observed worldwide, with a rise in testicular cancer the first trend to be noted. As will be discussed in detail in this review, studies on the origin and biology of the early stage of this neoplasia played a key role for the understanding of the association between male reproductive disorders and their possible link to changing environment and lifestyle.

A bit of history: histopathology of germ cell neoplasia

Germ cell tumours have fascinated several generations of pathologists because of their histological heterogeneity and seemingly unlimited ability to differentiate into all somatic tissues (totipotency). Moreover, germ cell-like tumours were noticed in remote extragonadal locations, including intracranial sites, usually near the midline of the body. Histological complexity of germ cell tumours constituted a diagnostic conundrum and contributed to the chaos with numerous classifications and nomenclatures. Because classification is not the topic of this review, the readers are referred to specialist reviews and monographs (Grigor, 1993; Ulbright *et al*., 1999; Eble *et al*., 2004). An easy and logical division of testicular germ cell tumours follows three age groups: tumours of newborns and infants (teratomas and yolk sac tumours), tumours of adolescents and young adults (seminomas and non-seminomas, which may also occur simultaneously as combined tumours) and the spermatocytic seminoma of elderly men (Oosterhuis and Looijenga, 2005). In addition, individuals with intersexual phenotype and dysgenetic gonads can harbour gonadoblastoma, a clinically benign but potentially malignant lesion (Scully, 1970). The tumours of infants and elderly are very rare.

One of the most important advances in the understanding of the biology and natural history of germ cell neoplasms, which led to a substantial revision of previous classifications, was the first description of testicular carcinoma *in situ* (CIS) in patients who subsequently developed testicular cancer, by a paediatric endocrinologist with a keen interest in testicular development and function in various pathologies (Skakkebæk, 1972). The cells described by Skakkebæk as a precursor for overt germ cell tumours were seen previously, however, others did not recognize their biological significance and considered them as 'degenerate forms' secondary to a tumour or 'intratubular spread of tumour cells' (Azzopardi *et al*., 1961; Mark and Hedinger, 1965), even several years after the Skakkebæk's description of CIS (Teilum, 1976; Pugh and Parkinson, 1981). Skakkebæk himself acknowledged those earlier descriptions (Skakkebæk, 1981), but it required an intervention by Gondos (1990) and a recent gracious commentary by Parkinson and Harland (2002) to put the earlier history of the discovery of CIS in the correct context. After a few years of denials and discussions, CIS has been commonly accepted as a precursor for all germ cell tumours of the adolescents and young adults, both seminomas and non-seminomas (Ulbright *et al*., 1999). Other synonyms for CIS have been proposed: intratubular germ cell neoplasia (ITGCN), also called unclassified (ITGCNU) (Ulbright *et al*., 1999), testicular intraepithelial neoplasia (Loy and Dieckmann, 1990) and gonocytoma *in situ* (Grigor, 1993). As will be evident from the discussion below, the last term may be the most accurate from the biological point of view.

Already some of the early studies of Skakkebæk and his group provided evidence that CIS was the pre-invasive lesion for the tumours of the adolescents and young adults but not for the infantile tumours or spermatocytic seminoma (Müller *et al*., 1987; Skakkebæk *et al*., 1987; Jørgensen *et al*., 1995a). Biological differences in the pathogenesis of these rare tumours have been confirmed subsequently by studies of genomic aberrations and gene expression patterns (Hawkins *et al*., 1997; Kraggerud *et al*., 1999; Perlman *et al*., 2000; Schneider *et al*., 2001; Stoop *et al*., 2001; Rajpert-De Meyts *et al*., 2003b; Looijenga *et al*., 2006).

Phenotypic features of CIS in relation to germ cell differentiation

Morphological features of CIS cells (Figure 1) have been described in numerous previous articles and pathology textbooks (Skakkebæk, 1972; Ulbright *et al*., 1999; Rørth *et al*., 2000; Eble *et al*., 2004). Close morphological similarity between CIS cells and human fetal gonocytes (as well as seminoma cells and the neoplastic germ cells of gonadoblastoma) was noticed soon after the first description of this lesion and later confirmed by ultrastructural studies (Holstein and Körner, 1974; Nielsen *et al*., 1974; Gondos, 1993). Subsequent studies provided supporting evidence for these similarities based on the comparison of immunohistochemical markers (Hustin *et al*., 1987; Jørgensen *et al*., 1993, 1995b, 1997; Honecker *et al*., 2004). Over the years, more and more proteins/antigens were identified in CIS cells (a partial list is presented in Table I). The status of knowledge on the emerging phenotype of the CIS cell up to year 2002 was summarized in my previous review (Rajpert-De Meyts *et al*., 2003a). Here, only the most important earlier findings are briefly highlighted, whereas the most recent advances are described in greater detail.

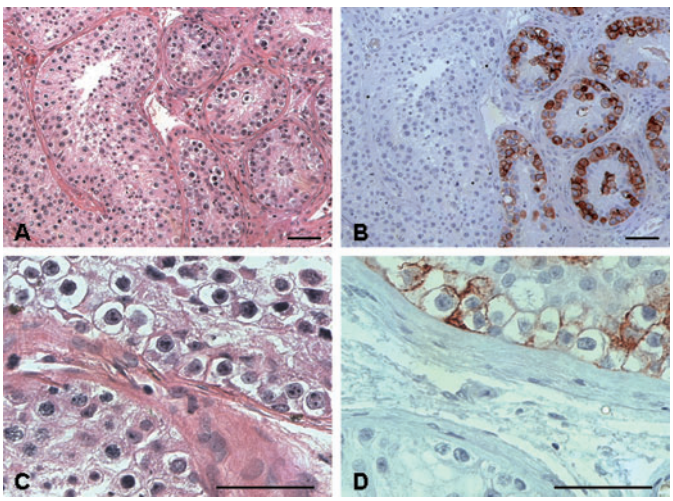

Figure 1. Histological appearance of human adult testis with carcinoma *in situ* (CIS), in cross-sections stained with haematoxylin–eosin (on the left). CIS cells are visualized by immunohistochemical staining for placental-like alkaline phosphatase (PLAP) in the same tissue samples (images on the right). (**A** and **B**) Low power images showing the different appearance of tubules with CIS in comparison with tubules with preserved spermatogenesis. (**C** and **D**) Higher magnification images showing details of CIS cells' morphology. Scale bar, 50 μm.

Table I. A list of selected proteins/antigens, which are expressed in carcinoma *in situ* (CIS) cells, presented in relation to the expression pattern in normal human male germ cells during their differentiation and maturation and in overt testicular germ cell tumours

Protein/antigen (gene)	ESC	PGC	Gonocytes	Sp-gonia	Sp-cytes	Sp-tids	CIS	SEM	N-SEM		SpSEM
									EC	TER	
NANOG	+	+	+	–	–	–	+	+	+	–	–
OCT3/4 (*POU5F1*)	+	+	+	–	–	–	+	+	+	–	–
AP-2γ (*TFAP2C*)	+	+	+	–	–	–	+	+	+	–/+	–
TRA-1-60	+	+	+/–	–	–	–	+/–	+/–	+	–	–
PLAP *(ALPL)*	–	+	+	–	–	–	+	+	+/–	–	–
M2A *(PDPN)*	?	+	+	–	–	–	+	+	–	–	–
KIT	+	+	+/–	–/+	–	–	+	+	–	–	–
DAZL1	?	+	+	+/–	+	–	+	+/–	–	–	?
VASA	?	+/–	+/–	+	+	+	+	+/–	–	–	+
Hiwi	?	+	+	+	+	+/–	+	+/–	–	–	?
TSPY	?	?	+	+	–	–	+	+	–	–	–
Cyclin D2 (*CCND2*)	?	?	+	–	–	–	+	+/–	+/–	+/–	+?
MAGE-A4	?	–	+	+	+/–	–	+/–	+/–	–	–	+
NY-ESO-1	?	–	+	+	+	–	+/–	–	–	–	+/–

EC, embryonal carcinoma; ESCs, embryonic stem cells; N-SEM, non-seminoma; PGC, primordial germ cells; SEM, seminoma; Sp-cytes, spermatocytes; Sp-gonia, spermatogonia; SpSEM, spermatocytic seminoma; Sp-tids, spermatids; TER, teratoma.
A strong expression is marked by +, a heterogeneous expression by +/-. A minus sign means that a protein is not detectable by immunohistochemistry, but it may be present in a given cell type in extremely low quantities, and the gene may be highly expressed at the RNA level. A question mark means that there is no information concerning the protein presence. Modified and updated from Rajpert-De Meyts *et al*. (2003a).

CIS markers, including the KIT receptor, are also expressed in human gonocytes

Early studies focussed on finding clinically useful marker to facilitate the detection of CIS in testicular biopsies. A classic example is placental-like alkaline phosphatase (PLAP, Figure 1), the first identified marker of murine primordial germ cells (PGCs) with still unknown biological function, which remains to this day the most commonly used marker for CIS and seminoma in testicular biopsies and other pathological tissue samples (Jacobsen and Nørgaard-Pedersen, 1984; Hustin *et al*., 1987; Rajpert-De Meyts *et al*., 2003a; references therein).

Over the years, the list of markers for CIS steadily grew; the early markers were usually identified serendipitously, e.g. by testing of an antibody against a glycoprotein abundant in a tumour cell line. Two of these markers, TRA-1-60 (Giwercman *et al*., 1993; Badcock *et al*., 1999) and M2A (Giwercman *et al*., 1988; Marks *et al*., 1999), which are abundant in CIS but undetectable in the normal adult testis, were detected in normal fetal and infantile germ cells, thus giving the first evidence supporting the hypothesis of the prenatal origin of CIS (Jørgensen *et al*., 1993, 1995b).

Further evidence for our hypothesis was provided by investigations of the expression of *c-KIT* in germ cell neoplasms. This gene encodes a cell membrane tyrosine kinase receptor for stem cell factor, a signalling system essential for early germ cell survival, as was first observed in mutant mice with either *W* or *Sl* phenotype (Chabot *et al*., 1988; Huang *et al*., 1990; Yarden *et al*., 1987). Differential expression of *KIT* was first described in germ cell tumours by Strohmeyer *et al*. (1991a) and detected in CIS cells (Figure 2) by Rajpert-De Meyts and Skakkebæk (1994), followed by several other studies (Izquierdo *et al*., 1995; Strohmeyer *et al*., 1995; Bokemeyer *et al*., 1996). As expected, KIT was also strongly expressed in fetal and infantile gonocytes (Jørgensen *et al*., 1995b; Robinson *et al*., 2001; Gaskell *et al*., 2004; Honecker *et al*., 2004) but very low or undetectable in adult spermatogonia in the adult human testis, although this has been somewhat dependent on the specificity of the antibodies and tissue fixation used (Rajpert-De Meyts *et al*., 2003b). The ontogeny of expression of KIT in the human testis demonstrated that it is present at a very high level in the majority of gonocytes during the first trimester of gestation, thereafter the KIT expression was gradually down-regulated (Jørgensen *et al*., 1995b; Gaskell *et al*., 2004; Honecker *et al*., 2004). The retention of a very high expression of KIT beyond a normal window was noted in dysgenetic fetal gonads of some intersex cases (Rajpert-De Meyts *et al*., 1996a). As KIT is a potent pro-survival factor, its prolonged expression could give a growth advantage to the surviving undifferentiated cells. This observation, along with a known association of CIS with poor gonadal development (Table II), led to a new hypothesis that a delay in differentiation could be of one of the mechanisms of neoplastic transformation of germ cells (Rajpert-De Meyts *et al*., 1998a). This is in the line with reports on 'gain-of-function' mutations in the *c-KIT* gene in virtually all sporadic bilateral tumours, both seminomas and non-seminomas (Looijenga *et al*., 2003a), and in a subset of familial and sporadic unilateral testicular tumours but, interestingly, less frequently in non-seminomas (Tian *et al*., 1999; Madani *et al*., 2003; Kemmer *et al*., 2004; Rapley *et al*., 2004). The high frequency of mutations of KIT in bilateral tumours suggests that the mutations most probably had occurred in PGCs, before their migration to the gonadal regions has taken place (Looijenga *et al*., 2003a).

Stem cell-like features: is CIS a fossil from the embryonic past?

The high expression of KIT (the receptor for the stem cell factor), which is present in different types of tissue-specific stem cells, turned our attention into stem cell-like characteristics of CIS

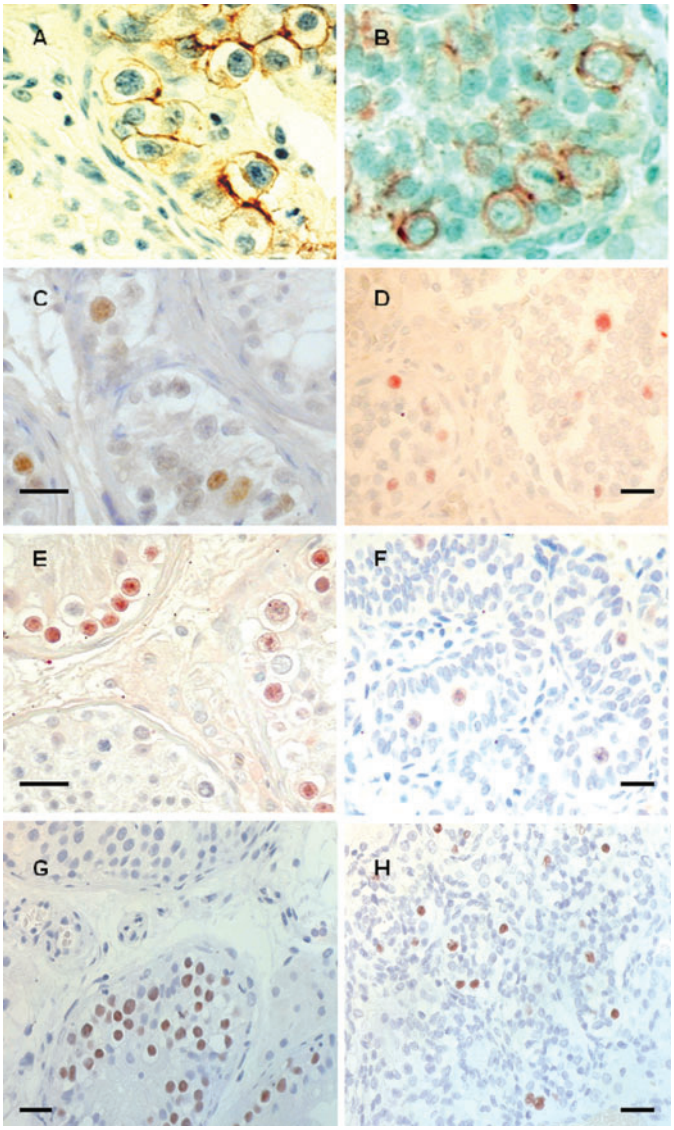

Figure 2. Examples of immunohistochemical staining for proteins highly expressed in carcinoma *in situ* (CIS) cells in adult testicular specimens (left) and in fetal gonocytes in normal fetal testes (right). Scale bar, 20 µm. (**A** and **B**) KIT, (**C** and **D**) p53, (**E** and **F**) OCT-4 and (**G** and **H**), AP-2γ.

cells. Previous studies of embryonal carcinoma-derived cell lines have demonstrated that they closely resemble human ESCs, including such hallmark features, as pluripotency and ability to differentiate when stimulated with retinoic acid (Andrews, 1984, 1998). Among the above-mentioned early markers for CIS cells was TRA-1-60, one of the best known markers for embryonal carcinoma and human ESC (Andrews *et al*., 1984; Giwercman *et al*., 1993; Badcock *et al*., 1999; Henderson *et al*., 2002; Park *et al*., 2004). More recently, OCT-4 (or OCT-3/4) encoded by *POU5F1*, the first transcription factor associated with pluripotency and specific for ESC (Schöler *et al*., 1989) was detected in CIS cells, gonadoblastoma and overt germ cell tumours, with the exception of differentiated teratomas (Palumbo *et al*., 2002; Gidekel *et al*., 2003; Looijenga *et al*., 2003b; Jones *et al*., 2004; Rajpert-De Meyts *et al*., 2004). Interestingly, OCT-4 was highly expressed by virtually all CIS cells in all these studies, whereas other markers, TRA-1-60 and to lesser extent KIT, were present in a subset of CIS cells only, preferentially in those in the vicinity of non-seminomas or seminomas, respectively, thus demonstrating a remarkable heterogeneity of CIS cells (Rajpert-De Meyts *et al*., 1996b). Heterogeneity of the expression of certain embryonic and germ cell-specific markers in CIS cells indicates plasticity of the phenotype of CIS cells, which may begin invasive transformation while still *in situ*.

Recent development of high throughput methods sped up markedly the characterization of gene expression in germ cell tumours and CIS at the RNA level. Most of the published studies analysed gene expression profiles in overt tumours or tumour-derived cell lines, focusing first on genes on certain chromosomal regions, e.g. 17q and 12p (Skotheim *et al*., 2002; Rodriguez *et al*., 2003), and later on a genome-wide analysis (Okada *et al*., 2003; Sperger *et al*., 2003; Skotheim *et al*., 2005). The Norwegian group investigated also gene expression at the protein level in a large array of tissues, including CIS, and confirmed the expression of *JUP* (plakoglobulin) in all CIS samples studied (Skotheim *et al*., 2003).

The first study that focussed on the expression profile of CIS (Hoei-Hansen *et al*., 2004a) used differential display and identified several genes that function in fetal life and thus supported the hypothesis of fetal origin of CIS. A substantial advance was the study by Almstrup *et al*. (2004), which using a genome-wide cDNA microarray, identified a large number of genes not previously reported in CIS. Importantly, the gene expression profile of CIS revealed a remarkable similarity to ESC (Almstrup *et al*., 2004). Among the genes over-expressed in CIS were *NANOG*, *POU5F1* (OCT-3/4), *KIT*, *SFRP1*, *TFAP2C* and several members of the *DPPA* family, which all have been identified in human ESC (Sato *et al*., 2003; Sperger *et al*., 2003; Clark *et al*., 2004), and more recently, also in embryonal carcinoma (Skotheim *et al*., 2005). A more detailed analysis of NANOG in CIS and germ cell tumours demonstrated a pattern of expression essentially identical to that of OCT-3/4 (Hart *et al*., 2005; Hoei-Hansen *et al*., 2005b). A common feature of these genes is their link to pluripotency; they prevent further differentiation of the cell and ensure a 'stock' of undifferentiated cells to renew the tissue. Outside the early embryonic development, NANOG and OCT-3/4 are only found in immature germ cells. A high expression of these genes is a probable explanation of the ability of CIS cells to undergo reprogramming to pluripotent embryonal carcinoma and further differentiation to teratomas, which may contain all types of somatic tissues.

Some of the genes associated with 'stemness' are present not only in ESC but also in various tissue-specific stem cells, e.g. *KIT* and *TFAP2C*. *TFAP2C* (mapped to chromosome 20q13.2), which encodes the transcription factor activator protein-2 (AP-2γ), was previously known as a possible oncogenic factor in other neoplasms, e.g. breast cancer (Turner *et al*., 1998) but never detected in testis. We established AP-2γ as a novel marker for fetal gonocytes and neoplastic germ cells, including testicular CIS (Figure 2), with a role in pathways regulating cell differentiation and a possible involvement in testicular oncogenesis (Hoei-Hansen *et al*., 2004b). This was confirmed by another study (Pauls *et al*., 2005). Thanks to its abundance in nuclei of CIS cells; AP-2γ is currently under investigation as a possible tool for the identification of CIS cells in semen samples in a clinical setting (Hoei-Hansen *et al*., 2005a).

Studies of the pattern of expression during development (Figure 3) demonstrated that OCT-4, AP-2γ, NANOG, as well as KIT, and

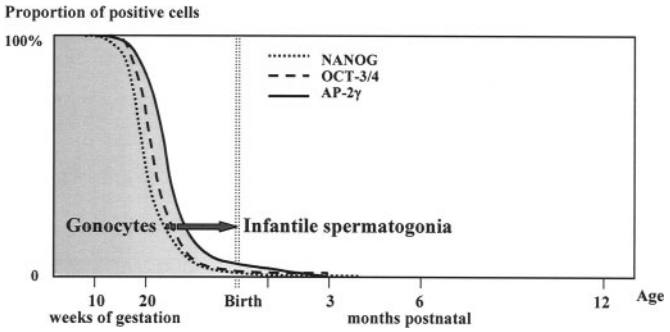

Figure 3. Developmental pattern of the expression of three markers of carcinoma *in situ* (CIS): NANOG, OCT-3/4 (POU5F1) and AP-2γ. The image shows approximately smoothed curves based on the combined results of several studies (Gaskell *et al*., 2004; Hoei-Hansen *et al*., 2004b, 2005b; Honecker *et al*., 2004; Rajpert-De Meyts *et al*., 2004).

probably a number of other CIS markers are abundant in early fetal gonocytes and the expression gradually decreases while gonocytes differentiate to infantile spermatogonia (Jørgensen *et al*., 1995b; Gaskell *et al*., 2004; Hoei-Hansen *et al*., 2004b, 2005b; Honecker *et al*., 2004; Rajpert-De Meyts *et al*., 2004). During human fetal testicular development, a rapid transition from PGCs (which in the testis are germ cells not yet enclosed in seminiferous cords) to gonocytes first takes place, later followed by much slower differentiation of gonocytes into pre-spermatogonia (also called infantile spermatogonia). At that time, germ cells gradually loose their embryonic characteristics while acquiring features of germ cells manifested by the expression of male-specific genes. It is important to underline here the continuum of the expression profile of germ cells, which are the only cell type in the body that retains for such a long time the high expression of genes necessary to maintain ESC-like pluripotency.

Germ cell-specific genes

In addition to ESCs and early fetal germ cells, CIS cells have also a lot in common with normal germ cells of the adult testis. Numerous of proteins/antigens present in normal spermatogonia were also found in CIS cells. The list of such proteins is growing practically by the day. Among the first published were globotriazol ceramide, Gb3 (Kang *et al*., 1995), and neuron-specific enolase, NSE (Kang *et al*., 1996), followed by many others, including some found also in spermatocytes and even in haploid spermatids, as listed in Table I (and reviewed in Rajpert-De Meyts *et al*., 2003a). One recent example is *VASA*, a gene-encoding DEAD-box RNA helicase, which is present in human germ cells throughout their development and maturation (Castrillon *et al*., 2000; Honecker *et al*., 2004) and is also expressed in CIS and overt tumours that retain germ cell-like morphology, such as testicular seminomas and ovarian dysgerminomas (Zeeman *et al*., 2002).

Recent advances in studies on germ cells uncovered a large number of genes that are germ cell-specific, but their biological function has not yet been elucidated, except that many of these genes appear to be involved in RNA processing and regulation, which is essential for spermatogenesis. As expected, quite a few of male germ cell-specific genes are located on the Y chromosome (Lahn and Page, 1997). Very little is known about the expression and function of these genes during early development of germ cells and even less about possible changes in testicular dysgenesis. An early study reported the expression of *RBMY* gene family both in the fetal and in the adult testis (Elliot *et al*., 1997), however, in more recent studies, RBMY was not detected by immunohistochemistry neither in CIS cells nor in overt tumours (Lifschitz-Mercer *et al*., 2000; Schreiber *et al*., 2003). Whether or not down-regulation of this gene family has something to do with neoplastic transformation of early germ cells into CIS remains to be elucidated. Another germ cell-specific gene family includes *DAZ* (on the Yq, usually consist of four copies) and closely related autosomal genes *DAZL* and *BOULE*. DAZ and DAZL have been described in mitotic germ cells, including PGCs and gonocytes (Reijo *et al*., 2000; Xu *et al*., 2001). Consequently, DAZL protein was detected in CIS, in seminomas but not in non-seminomas, consistent with its germ cell-specific function (Lifschitz-Mercer *et al*., 2002). Another multicopy gene, *TSPY*, was suggested as a candidate gene for gonadoblastoma (Salo *et al*., 1995; Tsuchiya *et al*., 1995). *TSPY* in the adult testis is expressed in spermatogonia, and its protein product was also described in immature germ cells in undifferentiated tubules of dysgenetic testes, CIS, seminoma (Schnieders *et al*., 1996) and gonadoblastoma (Lau *et al*., 2000; Kersemaekers *et al*., 2005). The function and biological role of TSPY remains to be elucidated. Likewise, it remains to be proven that *TSPY* is the only gene responsible for gonadoblastoma, as this tumour is frequently seen in mixed gonadal dysgenesis where there is a mosaic aneuploidy of sex chromosomes (46,XY/45,X). The presence of gonadoblastoma is thus most probably a result of male germ cells developing in an insufficiently masculinized gonad because of the lack of function of the Y-chromosome genes in somatic cells in the vicinity. As it will be discussed further, a similar pathogenesis is most probably responsible for CIS, except that CIS occurs in testes with development impaired to much lesser degree than is the case in mixed gonadal dysgenesis.

According to traditional knowledge, genes on the Y chromosome were considered to play the principal role in male reproduction, whereas the X chromosome was more linked to the female fertility. Female ovarian failure is frequently caused by the monosomy (Turner syndrome) or deletions of the X chromosome (reviewed in Zinn and Ross, 2001; Laml *et al*., 2002; Schlessinger *et al*., 2002). Recent years provided new evidence that the X chromosome contains a large number of genes expressed in male germ cells and is apparently essential not only for the female but also for the male germ cell function (Wang *et al*., 2001; Wang, 2004). Only a few of these genes have been studied so far in germ cell neoplasms. Of particular interest is large family of the so-called ‘cancer/testis’ genes, most of them mapped to the X chromosome, which were given this name because—apart from germ cells—they were only detected in various somatic cancers, e.g. melanoma and breast cancer (reviewed in Scanlan *et al*., 2002). Two members of this family, MAGE-A4 and NY-ESO-1, are highly expressed at the protein level in normal fetal gonocytes at the transition period to infantile pre-spermatogonia, in adult spermatogonia as well as in a subset of CIS cells and germ cell tumours, including in spermatocytic seminoma but not in non-seminomas (Jungbluth *et al*., 2000; Aubry *et al*., 2001; Yuasa *et al*., 2001; Satie *et al*., 2002; Rajpert-De Meyts *et al*., 2003b). Such a pattern of expression is consistent with a physiological

function of these genes in germ cells, in analogy to the above-mentioned germ cell-specific genes of the Y chromosome. The lack of expression of MAGE-A4 and NY-ESO-1 in non-seminomatous tumours is poorly understood but may be explained by differences in the genome methylation, which is much more pronounced in non-seminomas (Koul *et al*., 2002; Smith-Sorensen *et al*., 2002; Smiraglia *et al*., 2002; Honorio *et al*., 2003). The re-expression of cancer/testis genes in somatic tumours is probably also linked to changes in DNA methylation of promoter regions (Maio *et al*., 2003) but may be a result of other regulatory mechanisms. The X chromosome is the most tightly controlled in this aspect because of the need to compensate for the double dosage effect in females. The process is controlled by the X-inactivation centre, which produces the *XIST* transcript, which in turn triggers chromatin changes by Polycomb group proteins and DNA methylation (Csankovszki *et al*., 2001; Heard, 2004). In male germ cells, *XIST* is transcribed, but the X chromosome remains largely active. Interestingly, the *XIST* transcript is also over-expressed in testicular germ cell tumours and in CIS cells, perhaps partly because of a frequent increase in the copy number of X chromosomes in aneuploid neoplastic germ cells (Looijenga *et al*., 1997; Kawakami *et al*., 2003; Hoei-Hansen *et al*., 2004a).

Studies of the cell cycle and DNA repair are consistent with the pre-meiotic origin of CIS

Profound differences in the biology of germ cell neoplasms in comparison with the somatic tumours are undoubtedly related to a very special feature of germ cells—their ability to switch from mitotic cell division to the meiotic division, which is required for gamete formation. Regulatory mechanisms involved in the two types of cell division differ, and a number of studies provided evidence supporting the pre-meiotic origin of germ cell tumours, including CIS. Cell division is a final step in the cell cycle, which has to be exquisitely regulated to maintain the balance between proliferation and differentiation, a disturbance of this balance may lead to cancer or cell death. Closely related to the cell cycle regulation are the mechanisms of DNA repair, which are essential to prevent cell death or neoplastic transformation, especially in cells subjected to adverse environmental effects. Germ cells appear to have inherently high sensitivity to cytotoxic drugs and irradiation. This feature is further magnified in germ cell-derived tumours (reviewed in Masters and Koberle, 2003; Spierings *et al*., 2003). This is, of course, with great benefit for the patients with germ cell neoplasms, who can be efficiently treated by cisplatin-based regimens (Einhorn, 1997) or, in certain cases of isolated CIS, even by irradiation alone (Von der Maase *et al*., 1986). The processes of DNA repair are regulated differently in mitotically dividing immature germ cells during testicular development, and different mechanisms are specifically triggered when the meiotic division starts at puberty, because the meiotic crossover requires double-strand DNA breaks. As far as CIS is concerned, the evidence accumulated so far unequivocally demonstrates that a high expression of the key tumour suppressors involved in the DNA repair, such as p53 (Bartkova *et al*., 1991) and CHK2 (Bartkova *et al*., 2001), is a persistent developmental feature. Both proteins are abundant in normal fetal gonocytes (see p53 in Figure 2); p53 is then down-regulated in spermatogonia, whereas CHK2 remains highly expressed in spermatogonia but disappears at the onset of meiosis (Quenby *et al*., 1999; Bartkova *et al*., 2001; Rajpert-De Meyts *et al*., 2003b). A recent study demonstrated that after the onset of meiosis, a rapid activation of the ATM kinase takes place in spermatocytes to process multiple DNA double-strand breaks (Bartkova *et al*., 2005).

A wealth of evidence indicates that the G1/S-phase transition of the cell cycle is primarily controlled by the retinoblastoma protein (pRB) pathway, which is commonly involved in the pathogenesis of various malignancies (Mihara *et al*., 1989; Bartek and Lukas, 2001; Sherr, 2004; references therein). The pRB pathway regulation appears to be different in germ cells and deregulated in germ cell tumours but without structural aberrations (mutations) typical for somatic cancers (reviewed in Bartkova *et al*., 2003b). The observed changes are most likely due to a direct transcriptional regulation, an increased promoter methylation, or a more recently discovered regulatory mechanism by micro-RNAs (reviewed in Ambros, 2001; Zamore and Haley, 2005). As far as the CIS cells are concerned, the first interesting observation was the lack of pRB in CIS, seminoma and embryonal carcinoma, with a normal expression in teratomas (Strohmeyer *et al*., 1991b). This surprising finding is consistent with developmental regulation of pRB, which is apparently physiologically down-regulated in fetal gonocytes but active in mature spermatogonia (Bartkova *et al*., 2003a). As pRB is a tumour suppressor, the lack of pRB in fetal germ cells and CIS may render these cells more vulnerable to oncogenic stimuli but simultaneously also more prone to apoptosis (Bartkova *et al*., 2003b).

The second interesting feature of CIS and overt germ cell tumours is the over-expression of a protooncogenic cyclin D2 (encoded by *CCND2* mapped to chromosome 12p), significance of which will be discussed below (Sicinski *et al*., 1996; Houldsworth *et al*., 1997; Bartkova *et al*., 1999; Schmidt *et al*., 2001). The third feature, important for our discussion on the origin of germ cell neoplasms in relation to the meiotic switch, is the lack of the cyclin-dependent kinase (CDK) inhibitor p19-INK4d in CIS and overt germ cell tumours. P19-INK4d is abundant in normal spermatocytes and detectable in spermatids but completely absent from fetal gonocytes (Bartkova *et al*., 2000). Similarly, cyclin A1—which was described in spermatocytes—has not been detected in CIS or seminomas (Liao *et al*., 2004). Taken together, the studies of the regulatory machinery of the cell cycle strongly support the origin of CIS from early fetal and pre-meiotic germ cells.

Genomic aberrations in CIS: 12p or not 12p?

The question addressed soon after the discovery of a remarkable resemblance of CIS cells and fetal germ cells was whether CIS cell is a truly neoplastic cell or simply an immature gonocyte persisting in an adult testis. While substantial knowledge concerning genomic aberrations of the overt germ cell tumours was accumulated, the studies of CIS lagged behind, mainly because of technical difficulties due to a low number of CIS cells, their relatively low rate of proliferation (Höfken and Lauke, 1996) and poor growth in culture (Rajpert-De Meyts *et al*., 1998b). Only after the advent of a new technology of the comparative genomic hybridization (Kallioniemi *et al*., 1992), the genome of CIS cells has been better characterized. A detailed overview of genomic aberrations in the germ cell neoplasms, including CIS, is beyond the scope of this article, therefore, the reader is referred to recent excellent review articles on this topic (Skotheim and Lothe, 2003; von Eyben, 2004). I shall discuss here only the aberrations that are

probably the most informative with regard to the possible mechanism of neoplastic transformation, namely polyploidization and regional amplification of chromosome 12p.

Like nearly all neoplasms, CIS cells found in the adults are aneuploid with a mean DNA content in the hyper-triploid to hypo-tetraploid range (Skakkebæk, 1972; Müller and Skakkebæk, 1981; de Graaff *et al*., 1992). The longest lasting controversy concerned the presence in CIS of an isochromosome of the short arm of chromosome 12, i(12p), an aberration first described by Atkin and Baker (1982) and considered a hallmark of overt germ cell tumours (Castedo *et al*., 1988; Rodriguez *et al*., 1992; Van Echten *et al*., 1995a). Even in germ cell tumours without apparent presence of i(12)p, some amplification of the 12p material have been reported (Castedo *et al*., 1988; Rodriguez *et al*., 1993; Suijkerbuijk *et al*., 1993). The i(12)p has usually identical arms and is probably caused by an erroneous centromeric division during mitotic anaphase (Sinke *et al*., 1993). However, some loci on 12q in i(12)p-positive tumours retain heterozygosity, and thus polyploidization has to precede the formation of i(12p) (Geurts van Kessel *et al*., 1989).

The i(12p) in CIS was sporadically demonstrated by karyotyping (Vos *et al*., 1990; Van Echten *et al*., 1995b), but this has been disputed as the majority of the subsequent molecular studies did not detect genomic amplification of that region in CIS (Rosenberg *et al*., 2000; Summersgill *et al*., 2001). It was, therefore, proposed that the formation of i(12p) was not involved in the early pathogenetic process, but the relative gain of 12p sequences was associated with survival of CIS independently of Sertoli cells leading to their transformation to invasive tumours (Looijenga *et al*., 2003c). Our own study performed on the microdissected CIS cells by the comparative genomic hybridization added a missing link in this puzzle: we demonstrated that there indeed was no gain of 12p in two cases of CIS found as an isolated pre-invasive lesion, however, a clear genomic amplification in this region was detected in nearly all cases of CIS present in the vicinity of invasive tumours (Figure 4), suggesting clonal heterogeneity and possibly genomic instability of CIS cells (Ottesen *et al*., 2003). A subsequent analysis performed on CIS cells flow-sorted according to the DNA ploidy (Ottesen *et al*., 2004a) supported a hypothesis first suggested by Oosterhuis *et al*. (1989, 1990) that the polyploidization (tetraploidization) probably precedes the gain of 12p and other chromosomal aberrations. Some allelic losses detected in CIS resemble quite closely those in seminoma and, to a lesser extent, those in non-seminomas (Faulkner *et al*., 2000). However, the pattern of chromosomal aberrations/imbalances in overt germ cell tumours reported in numerous studies is quite similar despite morphological differences among germ cell tumour types (reviewed in Van Echten *et al*., 1995a; Skotheim and Lothe, 2003; von Eyben, 2004). A recent analysis of a large number of germ cell tumour karyotypes proposed that a multipolar cell division with non-disjunction of a tetraploid precursor cell, combined with some secondary imbalances/structural changes, is the most likely model of the karyotypic evolution of germ cell tumours (Frigyesi *et al*., 2004). Overall, genetic evidence gathered so far supports the progression of these tumours from a polyploid precursor cell, such as CIS (Oosterhuis *et al*., 1989, 1990), but the mechanisms of polyploidization remain to be elucidated.

Why the gain of 12p is so interesting? A look at the list of genes located there explains that. A number of genes associated with pluripotency of ESC and human teratocarcinoma cell lines, e.g.. *NANOG*, *STELLAR*, *DPPA-5* and *GDF3* (Caricasole *et al*., 1998; Sato *et al*., 2003; Sperger *et al*., 2003; Clark *et al*., 2004; Skotheim *et al*., 2005), and with germ cell proliferation or increased survival, e.g. *CCND2* and *K-RAS* (Sicinski *et al*., 1996; Houldsworth *et al*., 1997; Roelofs *et al*., 2000), are localized to the 12p region. This region constitutes also one of the hot spots of highly expressed genes in the profiling study of CIS (Almstrup *et al*., 2004). Interestingly, non-random gains of chromosomal material in the same region have been reported in human ESC maintained for a prolonged period in culture (Draper *et al*., 2004). That study, and a more recent investigation by Maitra *et al*. (2005), reported also non-random aberrations in cultured ESC in 17q, a region frequently rearranged in germ cell tumours (Kraggerud *et al*., 2002; Skotheim *et al*., 2002) where a cluster of genes highly expressed in CIS was detected as well (Almstrup *et al*., 2004). The observation of chromosomal aberrations in cultured ESC indicates that the microenvironment of growing ESC may be important for genomic stability. The molecular mechanisms are though poorly understood, and it is not known whether 12p and 17q are especially sensitive to chromosomal rearrangements. An alternative hypothesis is that the genome of CIS cells undergoes many random aberrations, and only the aberrations that render the cells better adapted to a changed microenvironment survive. This hypothesis postulates that the regions 12p, 17q and probably parts of X harbour genes with oncogenic potential, perhaps particularly oncogenic for germ cells. Some of the genes in these regions are indeed highly expressed in CIS cells, and we listed these candidate genes in a recent review article (Almstrup *et al*., 2005). I speculate that a similarity between ESC and CIS could indicate that CIS cells perhaps may originate from PGCs or gonocytes through a similar mechanism of 'natural selection' of cells that adapted themselves to their disturbed microenvironment in the developing gonad. How the development of the early gonad may be disturbed is the matter discussed in the remaining part of this review.

Who is at risk for germ cell cancer? The importance of prenatal events and the concept of testicular dysgenesis syndrome

Conditions associated with germ cell cancer and factors which increase the risk of this cancer are numerous and surprisingly variable.

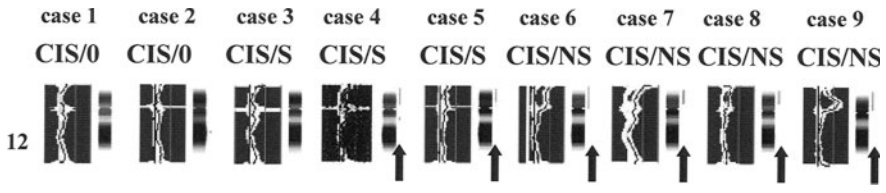

Figure 4. The mean ratio profiles of chromosome 12 analysed by comparative genomic hybridization in carcinoma *in situ* (CIS) cells microdissected from testes with CIS alone (CIS/0) or CIS adjacent to overt seminomas (CIS/S) or non-seminomas (CIS/NS). The relative gains in 12p regions are shown as light-grey vertical bars, marked with arrows on the right side of the ideograms of chromosomes (Ottesen et al., 2003; reprinted with permission from Wiley & Sons).

A systematic and critical analysis of clinical epidemiology of testicular cancer was recently published by Dieckmann and Pichlmeier (2004). Here, only a partial list of the best documented risk factors is listed in Table II, and a short summary of this topic is presented, mainly to illustrate the concept of the testicular dysgenesis syndrome (TDS).

Severe but relatively rare genetic abnormalities which cause testicular dysgenesis and the intersex syndrome (e.g. 45X/46XY and androgen insensitivity) are associated with a high risk of testicular cancer, often in combination with undescended testis and hypospadias (Aarskog, 1970; Scully, 1981; Savage and Lowe, 1990). Skakkebæk was the first to notice CIS in the dysgenetic testes of children with the intersex syndrome (Skakkebæk, 1979; Müller and Skakkebæk, 1984; Müller *et al*., 1985). Subsequently, several reports described the presence of CIS or gonadoblastoma in dysgenetic gonads of subjects with various forms of the intersex syndrome with or without structural aberrations of chromosomes (Cassio *et al*., 1990; MacMahon and Cussen, 1991; Rutgers and Scully, 1991; Jacobsen and Henriques, 1992; Ramani *et al*., 1993; Slowikowska-Hilczer *et al*., 2001; Slowikowska-Hilczer *et al*., 2003). In addition to linking gonadal dysgenesis with germ cell neoplasia, these observations support the notion that CIS and CIS-derived germ cell tumours may occur in the pre-pubertal testes and speak against an alternative hypothesis that the post-pubertal zygotene–pachytene spermatocyte is the cell of origin for CIS (Chaganti and Houldsworth, 2000).

Among more common urogenital abnormalities, cryptorchidism (undescended testis) is the best documented risk factor for testicular neoplasia, including CIS (Campbell, 1942; Morrison, 1976; Krabbe *et al*., 1979; Batata *et al*., 1982; Giwercman *et al*., 1989; Prener *et al*., 1996; Coupland *et al*., 1999; Weir *et al*., 2000). A recent meta-analysis evaluated the relative risk (RR) of testicular cancer in subjects with a history of cryptorchidism as 4.8 (95% CI = 4.0–5.7) (Dieckmann and Pichlmeier, 2004). There is also evidence for an association between testicular cancer and inguinal hernia or hypospadias (Morrison, 1976; Klein *et al*., 1996; Prener *et al*., 1996). Testes in cases with congenital urogenital malformations often are associated with some degree of maldevelopment, including clusters of poorly differentiated Sertoli-cell-only tubules and hyaline bodies (Sohval, 1954; Huff *et al*., 1993). More conspicuous but surprisingly common are histological signs of poor testicular development and function in adult patients with sporadic testicular tumours (Sohval, 1956), even in the seemingly 'normal' contralateral testes in patients with unilateral testicular cancer (Berthelsen and Skakkebæk, 1983; Hoei-Hansen *et al*., 2003). The degree of differentiation of Sertoli cells in adults with testicular cancer is variable depending on the grade of dysgenesis, but even morphologically immature Sertoli cells in most cases with complete spermatogenesis present elsewhere in the testis do not retain expression of the anti-Müllerian hormone, which is highly expressed before puberty (Rey *et al*., 1996; Rajpert-De Meyts *et al*., 1999). Hyaline bodies are frequently (but not always) seen on the ultrasound as testicular microlithiasis (reviewed in Holm *et al*., 2001). An association of microlithiasis with CIS and even testicular masses in the contralateral testis is so common that this ultrasonic abnormality should alert the attending physician about a possibility of testicular neoplasia, especially in patients with atrophic testes (Bach *et al*., 2003; Holm *et al*., 2003; de Gouveia Brazao *et al*., 2004).

Several studies documented that men with testis cancer had significantly reduced fertility before the development of their tumour, with a lower proportion of male children (decreased offspring sex ratio), and abnormal semen characteristics (Berthelsen and Skakkebæk, 1983; Møller and Skakkebæk, 1999; Jacobsen *et al*., 2000a,b; Richiardi *et al*., 2004c). On the contrary, men with subfertility have often a history of genital malformations and may harbour histological signs of testicular maldevelopment, including CIS, thus confirming an association between these conditions (Skakkebæk *et al*., 2003). Furthermore, an analysis of risk factors, such as low birthweight or intrauterine growth retardation (Depue *et al*., 1986; Morley and Lucas, 1987; Francois *et al*., 1997; Cicognani *et al*., 2002; English *et al*., 2003), suggested that the pathogenesis might be, at least partially, shared by germ cell tumours, cryptorchidism and male subfertility. Recently, a Norwegian study of risk factors for hypospadias found also, among others, a low birthweight and inguinal hernia (Aschim *et al*., 2004a). The epidemiological associations outlined above constituted the basis for a hypothesis of an aetiological link between the male reproductive disorders that are associated with impaired testicular development, within the so–called TDS presented schematically in Figure 5 (Skakkebæk *et al*., 2001; Asklund *et al*., 2004). The assumption that prenatal or perinatal factors are responsible for growing incidence of germ cell cancer and TDS is additionally corroborated by the birth cohort effects, meaning that the epidemiological trends are associated with the year of birth, and each subsequent cohort is more affected that the previous one. A birth cohort effect was, e.g., demonstrated for a decline in sperm concentrations of Scottish men (Irvine *et al*., 1996), one of the studies that followed the report on the possible decline of semen quality worldwide (Carlsen *et al*., 1992). One exception to the rule of the consecutive decline, which at the same time is a striking example of a birth cohort effect, was an unexplained decrease of the prevalence of testicular cancer among Scandinavian men born during wartime (Møller, 1993; Bergström *et al*., 1996).

A strong corroborating evidence for the TDS concept—which simultaneously incriminates environmental factors—is the geographical association between various components of TDS. A very illustrative example is given by the comparison of the rates in Denmark and in Finland, and another nearby located Nordic country. The incidence of testicular cancer, which is high in Denmark, is markedly lower in Finland (Adami *et al*., 1994; Richiardi *et al*., 2004a). Studies of the incidence rates of testicular cancer in populations migrating from these two countries to Sweden, which is

Table II. Risk factors for carcinoma *in situ* (CIS)/testicular cancer

Contralateral testis tumour
Cryptorchidism
Other genital malformations (inguinal hernia and hypospadias)
Intersex, including the androgen insensitivity syndrome
Gonadal dysgenesis
Familial testicular cancer
Testicular atrophy
Subfertility/infertility
Low birthweight
Down syndrome
Birth order (first pregnancy)
Early puberty
Estrogen excess during gestation

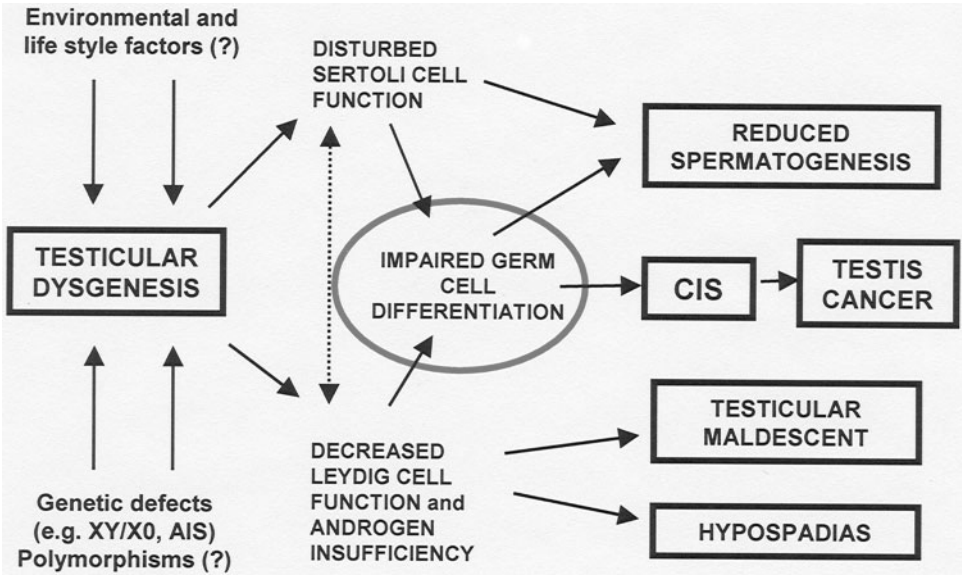

Figure 5. Schematic representation of the possible aetiology, pathogenesis and clinical manifestations of testicular dysgenesis syndrome emphasizing the key role of disturbed germ cell differentiation in the pathogenesis of testicular carcinoma *in situ* (CIS) (modified from Skakkebæk *et al*., 2001).

located in between, clearly demonstrated that the first generation immigrants retained the incidence as in their country of origin, whereas the second generation (born in Sweden) had the risk of testicular cancer similar to native Swedes (Hemminki and Li, 2002). Studies of semen quality found also all parameters better in Finland than in Denmark (Jensen *et al*., 2000; Jørgensen *et al*., 2001, 2002). The differences in rates of congenital genital malformations seemed also to be different, but less certain because of problems with definition and registry data (reviewed in Toppari *et al*., 2001). Data from other countries were confusing with some reporting an increase while other argued for a possible decline in cryptorchidism rates (Chilvers *et al*., 1984; Paulozzi, 1999; Toledano *et al*., 2003). Therefore, coordinated prospective studies of genital malformations have been launched in cohorts of infants, providing most telling evidence for the difference in the rates of cryptorchidism and hypospadias at birth in Denmark versus Finland (Boisen *et al*., 2004, 2005). At the same time, the Boisen *et al*. (2004) study demonstrated an increase of the incidence of cryptorchidism in Denmark over time (Buemann *et al*., 1961).

Geographical and ethnic differences have been noted much earlier for testicular cancer in other countries of the world, with unexplained high prevalence among Caucasians living in well-developed countries and notably lower prevalence among men of African descent and Asians, even inhabiting the same countries (English *et al*., 2003; Huyghe *et al*., 2003). The obvious question that arises is whether the reasons for the geographic and temporal differences in the prevalence of TDS are because of environmental differences or genetic variation/predisposition?

Genetic aspects of testicular cancer and TDS: can genetic polymorphisms explain geographic differences in the incidences?

Familial testicular cancer

Although familial testicular cancer is rare, this cancer has quite strong hereditary component. Sons and brothers of men with testicular cancer carry a four- and eight-fold increased risk of developing tumours, respectively (Lutke Holzik *et al*., 2004; references therein). However, a large proportion of familial cases, especially among brothers, may be explained, at least in part, by shared environment during early development (Hemminki and Li, 2004; Ottesen *et al*., 2004b). A few gene mutations have been reported in tumour tissues, but most of them have been linked to just one or two cases, with a notable exception of the activating mutation in the *KIT* gene, which has been detected in a subset of sporadic and familial tumours (Tian *et al*., 1999; Rapley *et al*., 2004) but which is present in virtually all bilateral testicular tumours (Looijenga *et al*., 2003a). So far, only one locus suspected for a germ cell cancer susceptibility gene has been reported at Xq27, but its importance is weakened by a simultaneous association with testicular maldescent (Rapley *et al*., 2000). Epidemiological observations suggest that most probably the majority of cases of testicular cancer are not because of a genetic mutation. Simple genetic polymorphisms, which are at the core of phenotypic diversity of human populations, may also be responsible for ethnic differences in the prevalence of human reproductive disorders. It is plausible that, e.g., genes that are involved in hormonal regulation of testicular development may contain polymorphic sequences that would slightly alter sensitivity to hormones, natural or synthetic. A similar phenomenon was observed in mice, where steroid hormones had vastly different effects in various mouse strains (Spearow *et al*., 1999). The human genotype is, of course, much more complicated that of an inbred laboratory mouse. Very few candidate genes relevant to humans have been studied so far, and the evidence is briefly reviewed here.

The androgen receptor

The androgen receptor gene is the most obvious candidate for a possible association of a polymorphism with disorders of male reproduction, in particular testicular cancer. Although the androgen function in the early fetal development has not been elucidated yet, the lack of function may impair genital development and increases

the risk of germ cell neoplasia considerably (Manuel *et al*., 1976; Quigley *et al*., 1995; Sultan *et al*., 2001). The opposite situation—an increased androgen signalling during development—may decrease the risk of testicular cancer (Rajpert-De Meyts and Skakkebæk, 1993). As mentioned above, the incidence of testicular cancer among Africans is very low, and by contrast they have a very high risk of prostate cancer, suggesting a possible role of higher testosterone levels *in utero* or other genetic predisposition (Henderson *et al*., 1988; Ross *et al*., 1998). One possible explanation could be a difference in the length of the polymorphic polyglutamine stretch in the androgen receptor, which is on average shorter among Africans and thus may be slightly more efficient in activating transcription (Sartor *et al*., 1999; Irvine *et al*., 2000).

The possible role of the two polymorphic trinucleotide (CAG and GGN) sequences, encoding polyglutamine and polyglycine stretches, has been extensively studied in all components of TDS. Expansion of the CAG repeat above 40 (normal range 8–37) causes spinal bulbar muscular atrophy, also known as the Kennedy syndrome, a serious neurodegenerative disease with progressive testicular atrophy and hypogonadism (La Spada *et al*., 1991). A series of studies, started by Tut *et al*. (1997) and Dowsing *et al*. (1999), reported a relation between the length of repeats and male infertility/subfertility while a similar number of studies failed to find such an association. It is impossible to cite in this review all studies investigating this problem in several centres around the world, but references can be found in meta-analyses and recent review articles (Rajpert-De Meyts *et al*., 2002a; Asatiani *et al*., 2003; Erasmuson *et al*., 2003; Ochsenkühn and de Kretser, 2003; Yong *et al*., 2003; Gottlieb *et al*., 2005). An association of the AR polymorphisms with testicular function (sperm production, sperm morphology and reproductive hormone profile)—both in infertile and fertile men—was also addressed by several studies (Mifsud *et al*., 2001; von Eckardstein *et al*., 2001; Härkönen *et al*., 2003; Milatiner *et al*., 2004). Most of these studies observed an inverse association between the number of CAG repeats and sperm production or quality. In our own study, a weak trend (not statistically significant) for a decrease in sperm concentrations with increasing $(CAG)_n$ was observed among fertile controls, but this trend disappeared after a greater number of subjects have been studied (Rajpert-De Meyts *et al*., 2002a). The whole issue remains open and debated; the reasons for the controversy include pathogenetic heterogeneity of clinical infertility, ethnical differences, poor characterization of control subjects in some studies and possible influences of confounding environmental factors. Possible differences in the mechanism of action of androgens within the testis in comparison with other parts of the male reproductive system should also be considered (Ochsenkühn and de Kretser, 2003).

Testicular cancer was investigated for the androgen receptor polymorphism in three studies only (Rajpert-De Meyts *et al*., 2002b; Giwercman *et al*., 2004; Garolla *et al*., 2005). Neither found an association of the cancer risk with the length of the CAG repeat alone, however, Giwercman *et al*. (2004) reported a possible link between the longest CAG repeats and the tumour progression to non-seminomas as well as clinically more aggressive disease, whereas Garolla *et al*. (2005) found that the combination CAG=20/GGC=17 was significantly more frequent in patients with testicular cancer than in controls. As far as genital malformations and undermasculinization are concerned, there is a better but not perfect consensus among the few published studies. Most of the European studies reported an association of these phenotypes with longer CAG (Lim *et al*., 2000, 2001) or GGN stretches or with certain combinations of CAG/GGN (Aschim *et al*., 2004b; Ferlin *et al*., 2005). By contrast, reports from Japan did not find any association, however, all these studies were performed by the same centre (Sasagawa *et al*., 2000; Ishii *et al*., 2001; Muroya *et al*., 2001), thus it would be beneficial for the final conclusion to have some confirmation from other Asian research groups.

Despite the controversy, a consensus slowly emerges that the AR-$(CAG)_n$ may play a role in the function of androgen-related pathways and their pathologies, especially outside the testis. However, this polymorphism should not be investigated in isolation, but a number of contributing factors (e.g. other diseases, lifestyle or environmental influence) should be considered (Hughes *et al*., 2001).

Possible role of deletions and polymorphisms of the Y chromosome

Individuals with the intersex syndrome and a relative reduction of the Y chromosome genetic material carry a high risk of germ cell neoplasia (Scully, 1981; Savage and Lowe, 1990; Peltomäki *et al*., 1991). The genes on the human Y chromosome that are most likely to be involved in germ cell differentiation and spermatogenesis are clustered in the so-called azoospermia factor (AZF) region of Yq11 (Tiepolo and Zuffardi, 1976; Vogt *et al*., 1992; Vogt, 1996), which is a part of the recently proposed male-specific Y-chromosome region (MSY) (Kuroda-Kawaguchi *et al*., 2001). This region is especially prone to interstitial deletions, which are associated with variable grade of testicular failure and impaired spermatogenesis and were first identified in infertile men (Tiepolo and Zuffardi, 1976; Vogt *et al*., 1992; Reijo *et al*., 1995; Vogt, 1996; Lahn and Page, 1997; Krausz *et al*., 2000; Kuroda-Kawaguchi *et al*., 2001; Frydelund-Larsen *et al*., 2002; Luetjens *et al*., 2002; Repping *et al*., 2002). It was long suspected that the propensity of the Yq region to those microdeletions may be caused by intrachromosomal recombination due to a presence of repetitive sequences, including those of ancient retroviruses, e.g. HERV (Kamp *et al*., 2000; Sun *et al*., 2000), high expression of which was reported in germ cell tumours (Herbst *et al*., 1996; Roelofs *et al*., 1998). Indeed, the sequencing of the entire Y chromosome and subsequent studies demonstrated that much of the sequence in MSY region consists of long palindromic repeats called amplicons, though most of them are not associated with retrotransposons (Kuroda-Kawaguchi *et al*., 2001; Tilford *et al*., 2001; Skaletsky *et al*., 2003). A deletion of a large amplicon usually removes a huge amount of DNA and is associated with very severe spermatogenic failure, with an exception of rare cases of subfertile men with large AZFc deletions who can occasionally produce sperm but still demonstrate testicular failure, manifested both by histological abnormalities and changes in the reproductive hormone profiles (Krausz *et al*., 2001a; Frydelund-Larsen *et al*., 2002).

More recently, smaller palindromes were discovered within the large amplicons in AZFc region. Deletions of these sequences remove several copies of multicopy gene families and are associated with a variable clinical and histological phenotype, although there is growing evidence that, e.g., gr/gr deletion may be a significant risk factor for decreased spermatogenesis, whereas b2/b3 is

probably neutral for testicular function (Fernandes *et al*., 2002; Repping *et al*., 2003, 2004; de Llanos *et al*., 2005; Giachini *et al*., 2005; Lynch *et al*., 2005). Some of these partial AZFc deletions, including gr/gr, can also be found in fertile men with normal spermatogenesis (Hucklenbroich *et al*., 2005), so it remains to be resolved whether these aberrations may play a role alone or only as a confounding factor predisposing to subfertility in the presence of other deleterious factors.

It is important to keep in mind that lack of recombination of the substantial part of the Y chromosome led to the formation of haplogroups which differ among populations, and these can contain single-nucleotide polymorphisms defining haplotypes (McElreavey and Quintana-Murci, 2003). A correlation between some of the Y-chromosome haplogroups and reduced sperm concentrations was found in Japan (Kuroki *et al*., 1999) and in Denmark (Krausz *et al*., 2001b). What is the mechanism leading to impaired spermatogenesis is not fully understood yet, but some haplotypes may segregate with rearrangements/inversions which may generate different types of the aforementioned partial AZFc deletions (Krausz *et al*., 2004; Machev *et al*., 2004). In some populations, e.g. Japan or Finland, certain Y chromosomes with partial AZFc deletions may have acquired compensatory mutations which would change the phenotype (Krausz *et al*., 2004; Vogt, 2005). This hypothesis is supported by observations from Finland, where there is no evidence of problems with spermatogenesis at the population level (Vierula *et al*., 1996; Jørgensen *et al*., 2002) despite a high prevalence of haplogroup N which is strongly associated with g1/g3 deletion (Krausz *et al*., 2004; Vogt, 2005).

The frequency of AZF deletions in the Danish population appears to be similar to that in other European countries and is not increased in patients with testicular cancer, thus the high prevalence of TDS in Denmark cannot be explained by a high incidence of such deletions (Krausz *et al*., 2001a; Frydelund-Larsen *et al*., 2003). A similar study was performed in Dutch patients and confirmed our observation of the absence of constitutional large AZF deletions in patients with testicular cancer (Lutke Holzik *et al*., 2005). This is also supported by the lack of association between Y lineages or haplotypes and testicular germ cell cancer (Quintana-Murci *et al*., 2003; Richard *et al*., 2004). Evidence gathered so far suggests that the molecular aetiology of TDS and sporadic testicular germ cell cancer most likely does not involve the same pathways as male infertility caused by deletions of genes located in the AZF region. A very recent study reported though that gr/gr deletions might confer susceptibility to the familial testicular germ cell cancer (Nathanson *et al*., 2005). However, the final conclusion awaits more detailed structural analysis of the Y chromosome in larger numbers of patients and controls.

Genes regulating testicular descent

As mentioned above in this review, failure of testicular descent (cryptorchidism) is a risk factor for testicular cancer, and the two disorders are probably closest associated with each other within the TDS. Aetiology of the majority of cases of cryptorchidism is unknown, indicating the involvement of a large number of factors in the pathogenesis of this complex disorder (Hutson *et al*., 1997). Among others, a lack of proper function of the androgen signalling pathways and anti-Müllerian hormone have been long known as capable of disturbing testicular descent, but more recently additional pathways have been unravelled. Detailed discussion on the genetic background of cryptorchidism is beyond the scope of this article, and the reader is referred instead to recent excellent reviews (Ivell and Hartung, 2003; Klonisch *et al*., 2004; Kolon *et al*., 2004). Here, only one pathway is briefly mentioned, that of insulin-like factor 3 (INSL3), as an example of a possible involvement of a genetic polymorphisms in the pathogenesis of some forms of TDS. INSL3, a testicular hormone (also known as relaxin-like factor, RLF), acts through a receptor named (G-protein-coupled receptor) LGR8/GREAT (Kumagai *et al*., 2002), and this system was first linked to testicular descent after targeted gene disruption in mice (Nef and Parada, 1999; Zimmerman *et al*., 1999; reviewed in Ivell and Bathgate, 2002). A large number of studies of the INSL3/LGR8 system in human subjects with cryptorchidism followed, some authors finding mutations, others failing to do so, but identifying several gene polymorphisms (Ferlin *et al*., 2003; references therein). Most of the identified mutations/polymorphisms were heterozygous, moreover in some cases the same genotype was linked to variable phenotypes, suggesting the involvement of multiple other factors, probably also environmental. Unravelling of the complex interplay between the structural changes of the INSL-3/LGR8 system and environmental impact on the regulatory pathways will require further studies, and this is only an example of one pathway. There are, undoubtedly, many more to investigate.

Are environmental or lifestyle factors responsible for increasing problems in male reproductive system including testicular cancer?

The rapid increase in the incidences of male reproductive problems indicates that environmental or lifestyle factors may play the primary role. A large number of epidemiological studies support this hypothesis. Probably the best documented and most illustrative are studies from Scandinavian countries, where excellent registries exist and where the reproductive problems were first noted. Among them, the finding of an association between a decreased incidence of testicular cancer and the year of birth, especially at wartime, clearly indicated the importance of external factors acting prenatally or perinatally (Møller, 1993; Bergström *et al*., 1996). This was supported by the aforementioned studies examining incidences of testicular cancer among migrating Scandinavian populations. (Hemminki and Li, 2002; Ekbom *et al*., 2003).

Which environmental or lifestyle factors can impair development of the reproductive system? Recent years provided growing evidence that the number and variability of contributing factors may be much greater than what we thought when the rise in testicular cancer was noted a few decades ago. The first hypothesis came from Henderson, and his group suggested a possible link between excessive exposure to bioavailable estrogens *in utero* (associated, e.g., with first pregnancy or maternal obesity) and reproductive abnormalities in men, in particular germ cell cancer (Henderson *et al*., 1979; Depue *et al*., 1983). The influence of parity was later confirmed by others (English *et al*., 2003; Richiardi *et al*., 2004b). Estrogens play an important role in spermatogenesis (Kula, 1988; Couse *et al*., 2001; Carreau *et al*., 2003), and a variant estrogen receptor β is present in fetal gonocytes (Gaskell *et al*., 2003), but the function of estrogens during early development of the testis is poorly understood. An ability to mimic estrogens and disturb hormonal pathways *in vitro* and *in vivo* in experimental animals

was discovered for a number of environmental chemicals, and endocrine hormone disrupters were suggested as possible inducers of reproductive problems in men (Sharpe and Skakkebæk, 1993; Toppari *et al*., 1996). However, the issue of endocrine disrupters appears to be very complex when viewed from the perspective of more recent evidence.

European epidemiological trends pointed at factors acting predominantly in highly developed countries, including those with an intensive agricultural industry, such as Denmark and Switzerland. Pollution of ground waters and food with potent synthetic hormones, e.g. estrogens, gestagens and androgenic anabolics used for meat production, has been suspected (Daxenberger *et al*., 2001). In addition, a large number of chemicals are components of pesticides, herbicides and food additives. For example, polychlorinated biphenyls, hexachlorobenzene and chlordanes elevated levels of which have been detected in blood from the mothers of men with testis cancer (Hardell *et al*., 2003). Studies of the mechanism of action of these compounds broadened the definition of endocrine disrupters to include chemicals interfering with various hormone pathways, most notably with androgen signalling and production (Gray *et al*., 2001; Williams *et al*., 2001). Recently, the attention of researchers has been focussed on phthalates, which are produced and utilized as plasticizers and softeners around the world in enormous quantities. Some phthalates, if administered *in utero*, can induce testicular dysgenesis and a TDS-like phenotypes in rats and rabbits (Foster *et al*., 2001; Fisher *et al*., 2003; Higuchi *et al*., 2003). The existing mechanistic evidence suggests that phthalates exert antiandrogenic effects (Fisher, 2004).

Apart from environmental chemicals, a host of lifestyle factors have also been indicated. Many of them are related to maternal habits, which may adversely influence the developing fetus, such as smoking (Jensen *et al*., 2004; Pettersson *et al*., 2004), maternal obesity and delayed childbearing (reviewed in Sharpe and Franks, 2002). The aetiology of testicular cancer and TDS is most likely multifactorial, and a relative importance of maternal factors and external exposures may be difficult to pinpoint.

Developmental model for the pathogenesis of CIS

Our current model of the pathogenesis of early stages of germ cell neoplasia is depicted schematically in Figure 6. Developmental arrest of germ cell differentiation is the core pathogenetic event leading to the origin of CIS. Most of this review discussed evidence indicating that CIS cells may be considered as transformed gonocytes. The initiation of the malignant transformation is most probably caused by the disturbance in the microenvironment of the differentiating fetal germ cells. Gonadal microenvironment during early development is very tightly regulated and exquisitely sensitive to hormones and paracrine factors. If this regulation is disturbed, a gonad may develop as a testis or as an ovary or something in between. Histological changes associated with CIS and evidence from animal models clearly indicate that somatic cells in the fetal testis, Sertoli and Leydig cells, or perhaps their precursors, are the mediators of hormonal and paracrine factors and are largely responsible for the differentiation of germ cells. However, direct influence on germ cells has also to be considered.

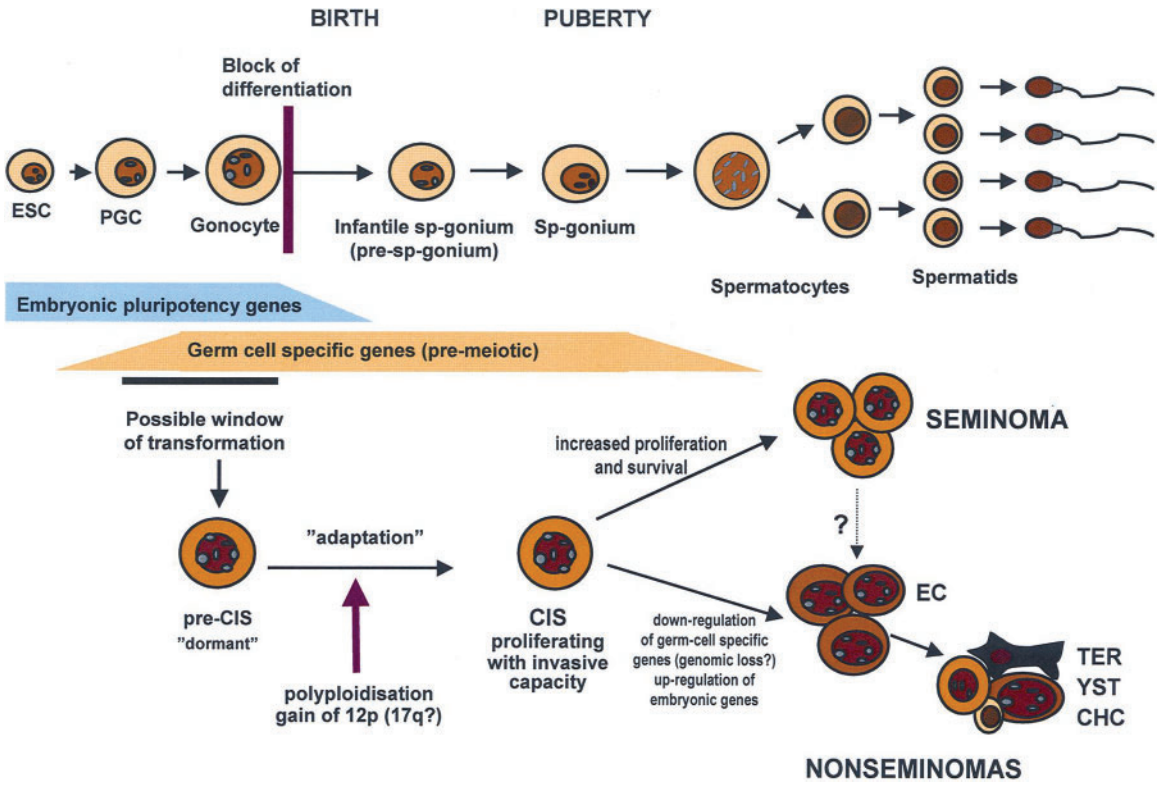

Figure 6. A scheme illustrating current understanding of the pathogenesis of testicular carcinoma *in situ* (CIS) in relation to germ cell differentiation. EC, embryonal carcinoma; TER, teratoma; YST, yolk sac tumour; CHC, choriocarcinoma.

In contrast to rodents, in the human fetus, differentiation of gonocytes into infantile spermatogonia is a relatively long and slow process. During this transition, embryonic traits disappear while germ cell-specific genes with a role in spermatogenesis are switched on (Figure 6). If something goes wrong and testicular differentiation is impaired, due to either an inherent genetic defect (e.g. in the androgen insensitivity syndrome) or an exposure to one or more environmental chemicals, this programme may be delayed or arrested, leading to the retention of embryonic features in germ cells outside the normal window of expression. Hormonal imbalance in the cellular microenvironment may lead to errors in mitosis–meiosis switch in 'sexually confused' germ cells and result in polyploidization. The mechanisms remain unknown, but we know that the processing of replicated sister chromatids and histone modification differ between mitosis and meiosis. One can speculate that a premature activation of some of the meiosis-specific mechanisms would somehow impair division of the replicated genome and cause polyploidization. Subsequently, other errors in cell division and progressive genomic aberrations would lead to further genomic instability and formation of transformed 'pre-CIS cells'. Most of these abnormal cells are probably eliminated, but some genomic changes may lead to the amplification of oncogenic pathways, which in combination with a high expression of anti-apoptotic pathways (e.g. KIT signalling) may favour survival of a subset of these cells. Recent observations in human ESCs undergoing chromosomal aberrations during long culture *in vitro* suggest that certain chromosomal gains (e.g. 12p, 17q or X) may be favourable for their adaptation and survival. A similar mechanism may be operating in transformed gonocytes *in vivo*; as mentioned earlier in this review, the pattern of chromosomal aberrations in CIS cells adjacent to overt tumours includes gains in the same chromosomal regions and may be responsible for their invasive ability. These adaptive changes and invasive progression are most probably triggered by the drastic change of testicular hormone production associated with puberty, since after puberty an explosive rise in the age-specific incidence of germ cell tumours is observed. Indeed, testicular germ cell tumours seem to be very rare in patients with severe hypogonadotrophic hypogonadism. Likewise, in patients with complete androgen insensitivity, undifferentiated gonocytes resembling CIS may persist for years without progressing to overt tumours (Manuel *et al*., 1976; Rutgers and Scully, 1991; Cools *et al*., 2005; Hannema *et al*., 2006).

Conclusions and future perspectives

The evidence summarized in this review demonstrates that testicular germ cell cancer is a developmental disease. Despite being manifested in young adults, this cancer is a result of disturbed gonadal development and germ cell differentiation. Our studies indicate that CIS cells are transformed gonocytes, which failed to differentiate and subsequently underwent non-random genomic aberrations facilitating their survival and further invasive progression, while retaining a high expression of embryonic genes linked to self-renewal and pluripotency. The ESC-like phenotype may explain the remarkable ability of CIS-derived tumours to differentiate to a variety of teratomatous somatic tissues. Further studies of the embryonic features of CIS cells and mechanisms of tumour progression may have broader implications for better understanding of basic pathways of PGC differentiation.

As shown in the Scandinavian countries, the prevalence of testicular cancer appears to be a good biomarker of testicular dysgenesis and may be used as a proxy to estimate the prevalence of other components of TDS in any given population. Differences between populations and between individuals within a given population may reflect differences in environment or lifestyle but may also be a result of genetic predisposition to reproductive problems. We have only begun to identify genes involved in the regulation of human gonadal development, and very few genes have so far been studied for possible polymorphisms. Environmental aetiological factors have not yet been elucidated, but testicular cancer and TDS are undoubtedly complex multifactorial diseases. To untangle this knot of possible factors will require further studies both at the level of populations and at the level of cells and their molecular pathways. Epidemiological trends in testicular cancer and TDS suggest that these disorders may be added to the list of the so-called 'civilization diseases', which includes diabetes, obesity and a number of cancers. These diseases increased around the world while the populations changed markedly their lifestyle and environment. However, the attention given to these diseases by researchers will hopefully elucidate their aetiology and provides tools for very early detection of negative trends and help to implement preventive measures.

Acknowledgements

I thank Professor Niels E. Skakkebæk for his generous support at every step of my research. I am grateful to many other colleagues who contributed to work reviewed here. The studies of our group were supported by the Danish Cancer Society, the Danish Medical Research Council, the Svend Andersen Foundation, The Research Council of Copenhagen University Hospitals (H:S), the Kirsten & Freddy Johannsen Foundation, the European Commission, the Vissing Foundation, the Carla Thiel Kragh Foundation and the Willumsen Foundation.

References

Aarskog D (1970) Clinical and cytogenetic studies in hypospadias. Acta Paediatr Scand Suppl 203,1–62.

Adami H, Bergström R, Möhner M, Zatonski W, Storm H, Ekbom A, Tretli S, Teppo L, Ziegler H, Rahu M et al. (1994) Testicular cancer in nine northern European countries. Int J Cancer 59,33–38.

Almstrup K, Hoei-Hansen CE, Wirkner U, Blake J, Schwager C, Ansorge W, Nielsen JE, Skakkebæk NE, Rajpert-De Meyts E and Leffers H (2004) Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. Cancer Res 64,4736–4743.

Almstrup K, Ottesen AM, Brask Sonne S, Hoei-Hansen CE, Leffers H, Rajpert-De Meyts E and Skakkebæk NE (2005) Genomic and gene expression signature of the pre-invasive testicular carcinoma in situ. Cell Tissue Res 322,159–165.

Ambros V (2001) MicroRNAs: tiny regulators with great potential. Cell 107,823–826.

Andrews PW (1984) Retinoic acid induces neuronal differentiation of a cloned human embryonal carcinoma cell line in vitro. Dev Biol 103,285–293.

Andrews PW (1998) Teratocarcinomas and human embryology: pluripotent human EC lines. APMIS 106,158–168.

Andrews PW, Banting GS, Damjanov I, Arnaud D and Avner P (1984) Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells. Hybridoma 3,347–361.

Asatiani K, von Eckardstein S, Simoni M, Gromoll J and Nieschlag E (2003) CAG repeat length in the androgen receptor gene affects the risk of male infertility. Int J Androl 26,255–261.

Aschim EL, Haugen TB, Tretli S, Daltveit AK and Grotmol T (2004a) Risk factors for hypospadias in Norwegian boys – association with testicular dysgenesis syndrome? Int J Androl 27,213–221.

Aschim EL, Nordenskjold A, Giwercman A, Lundin KB, Ruhayel Y, Haugen TB, Grotmol T and Giwercman YL (2004b) Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. J Clin Endocrinol Metab 89,5105–5109.

Asklund C, Jorgensen N, Kold Jensen T and Skakkebaek NE (2004) Biology and epidemiology of testicular dysgenesis syndrome. BJU Int 93,6–11.

Atkin NB and Baker MC (1982) Specific chromosome change, i(12p), in testicular tumours? Lancet 2,1349.

Aubry F, Satie A-P, Rioux-Leclercq N, Rajpert-De Meyts E, Spagnoli GC, Chomez P, De Backer O, Jegou B and Samson M (2001) *MAGE-A4*, a germ-cell specific marker is differentially expressed in testicular tumours. Cancer 902,2778–2785.

Azzopardi JG, Mostofi FK and Theiss EA (1961) Lesions of testes observed in certain patients with widespread chorioracinoma and related tumors. The significance and genesis of hematoxyllin-staining bodies in the human testis. Am J Pathol 38,207–225.

Bach AM, Hann LE, Shi W, Giess CS, Yoo HH, Sheinfeld J and Thaler HT (2003) Is there an increased incidence of contralateral testicular cancer in patients with intratesticular microlithiasis? AJR Am J Roentgenol 180,497–500.

Badcock G, Pigott C, Goepel J and Andrews PW (1999) The human embryonal carcinoma marker antigen TRA-1-60 is a sialylated keratan sulfate proteoglycan. Cancer Res 59,4715–4719.

Bartek J and Lukas J (2001) Pathways governing G1/S transition and their response to DNA damage. FEBS Lett 490,117–122.

Bartkova J, Bartek J, Lukas J, Vojtesek B, Staskova Z, Rejthar A, Kovarik J, Midgley CA and Lane DP (1991) p53 protein alterations in human testicular cancer including preinvasive intratubular germ cell neoplasia. Int J Cancer 49,196–202.

Bartkova J, Rajpert-De Meyts E, Skakkebæk NE and Bartek J (1999) D-type cyclins in adult human testis and testicular cancer: relation to cell type, proliferation, differentiation, and malignancy. J Pathol 187,573–581.

Bartkova J, Thulberg M, Rajpert-De Meyts E, Skakkebæk NE and Bartek J (2000) Lack of $p19^{INK4d}$ in human testicular germ-cell tumours contrasts with high expression during normal spermatogenesis. Oncogene 19,4146–4150.

Bartkova J, Falck J, Rajpert-De Meyts E, Skakkebæk NE, Lukas J and Bartek J (2001) Chk2 tumour suppressor protein in human spermatogenesis and testicular germ-cell tumours. Oncogene 20,5897–5902.

Bartkova J, Lukas C, Sørensen CS, Rajpert-De Meyts E, Skakkebæk NE, Lukas J and Bartek J (2003a) Deregulation of the RB pathway in human testicular germ cell tumours. J Pathol 200,149–156.

Bartkova J, Rajpert-De Meyts E, Skakkebæk NE, Lukas J and Bartek J (2003b) Deregulation of the G1/S-phase control in human testicular germ cell tumours. APMIS 111,252–266.

Bartkova J, Bakkenist CJ, Rajpert-De Meyts E, Skakkebæk NE, Sehested M, Lukas J, Kastan MB and Bartek J (2005) ATM activation in normal human tissues and testicular cancer. Cell Cycle 4,838–845.

Batata MA, Chu FCH, Hilaris BS, Whitmore WF and Golbey RB (1982) Testicular cancer in cryptorchids. Cancer 49,1023–1030.

Bergström R, Adami H, Mohner M, Zatonski W, Storm H, Ekbom A, Tretli S, Teppo L, Akre O and Hakulinen T (1996) Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. J Natl Cancer Inst 88,727–733.

Berthelsen JG and Skakkebaek NE (1983) Gonadal function in men with testis cancer. Fertil Steril 39,68–75.

Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto AM, Schmidt IM, Chellakooty M, Damgaard IN, Mau C, Reunanen M et al. (2004) Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 363,1264–1269.

Boisen KA, Chellakooty M, Schmidt IM, Kai CM, Damgaard IN, Suomi AM, Toppari J, Skakkebæk NE and Main KM (2005) Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth and reproductive hormone levels at 3 months of age. J Clin Endocrinol Metab 90,4041–4046.

Bokemeyer C, Kuczyk MA, Dunn T, Serth J, Hartmann K, Jonasson J, Pietsch T, Jonas U and Schmoll HJ (1996) Expression of stem-cell factor and its receptor c-kit protein in normal testicular tissue and malignant germ-cell tumours. J Cancer Res Clin Oncol 122,301–306.

Buemann B, Henriksen H, Villumsen AL, Westh A and Zachau-Christiansen B (1961) Incidence of undescended testis in the newborn. Acta Chir Scand 283,289–293.

Campbell HE (1942) Incidence of the malignant growth of the undescended testicle. A critical and statistical study. Arch Surg 44,353–369.

Caricasole AA, van Schaik RH, Zeinstra LM, Wierikx CD, van Gurp RJ, van den Pol M, Looijenga LH, Oosterhuis JW, Pera MF, Ward A et al. (1998) Human growth-differentiation factor 3 (hGDF3): developmental regulation in human teratocarcinoma cell lines and expression in primary testicular germ cell tumours. Oncogene 16,95–103.

Carlsen E, Giwercman A, Keiding N and Skakkebaek NE (1992) Evidence for decreasing quality of semen during past 50 years. BMJ 305,609–613.

Carreau S, Lambard S, Delalande C, Denis-Galeraud I, Bilinska B and Bourguiba S (2003) Aromatase expression and role of estrogens in male gonad: a review. Reprod Biol Endocrinol 1,35.

Cassio A, Cacciari E, D'Errico A, Balsamo A, Grigioni FW, Pascucci MG, Bacci F, Tacconi M and Mancini AM (1990) Incidence of intratubular germ cell neoplasia in androgen insensitivity syndrome. Acta Endocrinol (Copenh) 123,416–422.

Castedo SM, de Jong B, Oosterhuis JW, Seruca R, Idenburg VJ, Buist J and Sleijfer DT (1988) i(12p)-negative testicular germ cell tumors. A different group? Cancer Genet Cytogenet 35,171–178.

Castrillon DH, Quade BJ, Wang TY, Quigley C and Crum CP (2000) The human *VASA* gene is specifically expressed in the germ cell lineage. Proc Natl Acad Sci USA 97,9585–9590.

Chabot B, Stephenson DA, Chapman VM, 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.

Chaganti RSK and Houldsworth J (2000) Genetics and biology of adult human male germ cell tumours. Cancer Res 60,1475–1482.

Chilvers C, Pike MC, Forman D, Fogelman K and Wadsworth ME (1984) Apparent doubling of frequency of undescended testis in England and Wales in 1962-81. Lancet 2,330–332.

Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E and Cacciari E (2002) Low birth weight for gestational age and subsequent male gonadal function. J Pediatr 141,376–379.

Clark AT, Rodriguez RT, Bodnar MS, Abeyta MJ, Cedars MI, Turek PJ, Firpo MT and Reijo-Pera RA (2004) Human STELLAR, NANOG, and GDF3 genes are expressed in pluripotent cells and map to chromosome 12p13, a hotspot for teratocarcinoma. Stem Cells 22,169–179.

Cools M, van Aerde K, Kersemaekers AM, Boter M, Drop SL, Wolffenbuttel KP, Steyerberg EW, Oosterhuis JW and Looijenga LH (2005) Morphological and immunohistochemical differences between gonadal maturation delay and early germ cell neoplasia in patients with undervirilization syndromes. J Clin Endocrinol Metab 90,5295–5303.

Coupland CA, Chilvers CE, Davey G, Pike MC, Oliver RT and Forman D (1999) Risk factors for testicular germ cell tumours by histological tumour type. United Kingdom Testicular Cancer Study Group. Br J Cancer 80,1859–1863.

Couse JE, Mahato D, Eddy EM and Korach KS (2001) Molecular mechanism of estrogen action in the male: insights from the estrogen receptor null mice. Reprod Fertil Dev 13,211–219.

Csankovszki G, Nagy A and Jaenisch R (2001) Synergism of Xist RNA, DNA methylation, and histone hypoacetylation in maintaining X chromosome inactivation. J Cell Biol 153,773–784.

Daxenberger A, Ibarreta D and Meyer HDH (2001) Possible health impact of animal oestrogens in food. Hum Reprod Update 7,340–355.

de Gouveia Brazao CA, Pierik FH, Oosterhuis JW, Dohle GR, Looijenga LH and Weber RF (2004) Bilateral testicular microlithiasis predicts the presence of the precursor of testicular germ cell tumors in subfertile men. J Urol 171,158–160.

de Graaff W, Oosterhuis JW, De Jong B, Dam A, Van Putten WLJ, Castedo SMMJ, Sleijfer DT and Schraffordt Koops H (1992) Ploidy of testicular carcinoma in situ. Lab Invest 66,166–168.

de Llanos M, Ballesca JL, Gazquez C, Margarit E and Oliva R (2005) High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICSI candidates. Hum Reprod 20,216–220.

Depue RH, Pike MC and Henderson BE (1983) Estrogen exposure during gestation and risk of testicular cancer. J Natl Cancer Inst 71,1151–1155.

Depue RH, Pike MC and Henderson BE (1986) Birth weight and the risk of testicular cancer. J Natl Cancer Inst 77,829–830.

Dieckmann KP and Pichlmeier U (2004) Clinical epidemiology of testicular germ cell tumors. World J Urol 22,2–14.

Dowsing AT, Young EL, Clark M, McLachlan RI, de Kretser DM and Trounson AO (1999) Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. Lancet 354,640–643.

Draper JS, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J, Meisner L, Zwaka TP, Thomson JA and Andrews PW (2004) Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat Biotechnol 22,53–54.

Eble JN, Sauter G, Epstein JI and Sesterhenn IA (eds) (2004) Pathology and genetics of tumours of the urinary system and male genital organs. In

WHO Classification of Tumours, Vol. 7. International Agency for Research on Cancer, Lyon, pp. 1–395.
Einhorn LH (1997) Testicular cancer: an oncological success story. Clin Cancer Res 3,2630–2632.
Ekbom A, Richiardi L, Akre O, Montgomery SM and Sparen P (2003) Age at immigration and duration of stay in relation to risk for testicular cancer among Finnish immigrants in Sweden. J Natl Cancer Inst 95,1238–1240.
Elliott DJ, Millar MR, Oghene K, Ross A, Kiesewetter F, Pryor J, McIntyre M, Hargreave TB, Saunders PT, Vogt PH et al. (1997) Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y chromosome long arm. Proc Natl Acad Sci USA 94,3848–3853.
English PB, Goldberg DE, Wolff C and Smith D (2003) Parental and birth characteristics in relation to testicular cancer risk among males born between 1960 and 1995 in California (United States). Cancer Causes Control 14,815–825.
Erasmuson T, Sin IL and Sin FY (2003) Absence of association of androgen receptor trinucleotide expansion and poor semen quality. Int J Androl 26,46–51.
Faulkner SW, Leigh DA, Oosterhuis JW, Roelofs H, Looijenga LH and Friedlander ML (2000) Allelic losses in carcinoma in situ and testicular germ cell tumours of adolescents and adults: evidence suggestive of the linear progression model. Br J Cancer 83,729–736.
Ferlin A, Simonato M, Bartoloni L, Rizzo G, Bettella A, Dottorini T, Dallapiccola B and Foresta C (2003) The INSL3-LGR8/GREAT ligand-receptor pair in human cryptorchidism. J Clin Endocrinol Metab 88,4273–4279.
Ferlin A, Garolla A, Bettella A, Bartoloni L, Vinanzi C, Roverato A and Foresta C (2005) Androgen receptor gene CAG and GGC repeat lengths in cryptorchidism. Eur J Endocrinol 152,419–425.
Fernandes S, Huellen K, Goncalves J, Dukal H, Zeisler J, Rajpert-De Meyts E, Skakkebæk NE, Habermann B, Krause W, Sousa M et al. (2002) High frequency of *DAZ1/DAZ2* gene deletions in patients with severe oligozoospermia. Mol Hum Reprod 8,286–298.
Fisher JS (2004) Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reproduction 127,305–315.
Fisher JS, Macpherson S, Marchetti N and Sharpe RM (2003) Human 'testicular dysgenesis syndrome': a possible model using in-utero exposure of the rat to dibutyl phthalate. Hum Reprod 18,1383–1394.
Foster PMD, Mylchreest E, Gaido KW and Sar M (2001) Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update 7,231–235.
Francois I, De Zegher F, Spiessens C, D'Hooge T and Vanderschueren D (1997) Low birth weight and subsequent male subfertility. Pediatr Res 42,899–901.
Frigyesi A, Gisselsson D, Hansen GB, Soller M, Mitelman F and Hoglund M (2004) A model for karyotypic evolution in testicular germ cell tumors. Genes Chromosomes Cancer 40,172–178.
Frydelund-Larsen L, Krausz CG, Leffers H, Andersson A-M, Carlsen E, Bangsbøll S, McElreavey K, Skakkebæk NE and Rajpert-De Meyts E (2002) Inhibin B: a marker for the functional state of the seminiferous epithelium in patients with AZFc microdeletions. J Clin Endocrinol Metab 87,5618–5624.
Frydelund-Larsen L, Vogt P, Leffers H, Schadwinkel A, Daugaard G, Skakkebæk NE and Rajpert-De Meyts E (2003) No AZF deletion in 160 patients with testicular germ cell cancer. Mol Hum Reprod 9,517–521.
Garolla A, Ferlin A, Vinanzi C, Roverato A, Sotti G, Artibani W and Foresta C (2005) Molecular analysis of the androgen receptor gene in testicular cancer. Endocr Relat Cancer 12,645–655.
Gaskell TL, Robinson LL, Groome NP, Anderson RA and Saunders PT (2003) Differential expression of two estrogen receptor beta isoforms in the human fetal testis during the second trimester of pregnancy. J Clin Endocrinol Metab 88,424–432.
Gaskell TL, Esnal A, Robinson LL, Anderson RA and Saunders PT (2004) Immunohistochemical profiling of germ cells within the human fetal testis: identification of three subpopulations. Biol Reprod 71,2012–2021.
Geurts van Kessel A, van Drunen E, de Jong B, Oosterhuis JW, Langeveld A and Mulder MP (1989) Chromosome 12q heterozygosity is retained in i(12p)-positive testicular germ cell tumour cells. Cancer Genet Cytogenet 40,129–134.
Giachini C, Guarducci E, Longepied G, Degl'Innocenti S, Becherini L, Forti G, Mitchell MJ and Krausz C (2005) The gr/gr deletion(s): a new genetic test in male infertility? J Med Genet 42,497–502.
Gidekel S, Pizov G, Bergman Y and Pikarsky E (2003) Oct-3/4 is a dose-dependent oncogenic fate determinant. Cancer Cell 4,361–370.
Giwercman A, Marks A, Bailey D, Baumal R and Skakkebæk NE (1988) A monoclonal antibody as a marker for carcinoma in situ germ cells of the human adult testis. APMIS 96,667–670.
Giwercman A, Bruun E, Frimodt-Møller C and Skakkebæk NE (1989) Prevalence of carcinoma in situ and other histopathological abnormalities in testis of men with a history of cryptorchidism. J Urol 142,998–1001.
Giwercman A, Andrews PW, Jørgensen N, Müller J, Græm N and Skakkebæk NE (1993) Immunohistochemical expression of embryonal marker TRA-1-60 in carcinoma in situ and germ cell tumors of the testis. Cancer 72,1308–1314.
Giwercman A, Lundin KB, Eberhard J, Stahl O, Cwikiel M, Cavallin-Stahl E and Giwercman YL (2004) Linkage between androgen receptor gene CAG trinucleotide repeat length and testicular germ cell cancer histological type and clinical stage. Eur J Cancer 40,2152–2158.
Gondos B (1990) Re: carcinoma in situ of the testis. J Urol 144,1484–1485 [letter].
Gondos B (1993) Ultrastructure of developing and malignant germ cells. Eur Urol 23,68–75.
Gottlieb B, Lombroso R, Beitel LK and Trifiro MA (2005) Molecular pathology of the androgen receptor in male (in)fertility. Reprod Biomed Online 10,42–48.
Gray LE Jr, Ostby J, Furr J, Wolf CJ, Lambright C, Parks L, Veeramachaneni DN, Wilson V, Price M, Hotchkiss A et al. (2001) Effects of environmental antiandrogens on reproductive development in experimental animals. Hum Reprod Update 7,248–264.
Grigor KM (1993) A new classification of germ cell tumours of the testis. Eur Urol 23,93–103.
Hannema SE, Scott IS, Rajpert-De Meyts E, Skakkebæk NE, Coleman N and Hughes IA (2006) Testicular development in the complete androgen insensitivity syndrome. J Pathol 208,518–527.
Hardell L, van Bavel B, Lindstrom G, Carlberg M, Dreifaldt AC, Wijkstrom H, Starkhammar H, Eriksson M, Hallquist A and Kolmert T (2003) Increased concentrations of polychlorinated biphenyls, hexachlorobenzene, and chlordanes in mothers of men with testicular cancer. Environ Health Perspect 111,930–934.
Härkönen K, Huhtaniemi I, Mäkinen J, Hübler D, Irjala K, Koskenvuo M, Oettel M, Raitakari O, Saad F and Pöllänen P (2003) The polymorphic androgen receptor gene CAG repeat, pituitary-testicular function and andropausal symptoms in ageing men. Int J Androl 26,187–194.
Hart AH, Hartley L, Parker K, Ibrahim M, Looijenga LH, Pauchnik M, Chow CW and Robb L (2005) The pluripotency homeobox gene NANOG is expressed in human germ cell tumors. Cancer 104,2092–2098.
Hawkins E, Heifetz SA, Giller R and Cushing B (1997) The prepubertal testis (prenatal and postnatal): its relationship to intratubular germ cell neoplasia: a combined Pediatric Oncology Group and Children's Cancer Study Group. Hum Pathol 28,404–410.
Heard E (2004) Recent advances in X-chromosome inactivation. Curr Opin Cell Biol 16,247–255.
Hemminki K and Li X (2002) Cancer risks in Nordic immigrants and their offspring in Sweden. Eur J Cancer 38,2428–2434.
Hemminki K and Li X (2004) Familial risk in testicular cancer as a clue to a heritable and environmental aetiology. Br J Cancer 90,1765–1770.
Henderson BE, Benton B, Jing J, Yu MC and Pike MC (1979) Risk factors for cancer of the testis in young men. Int J Cancer 23,598–602.
Henderson BE, Bernstein L, Ross RK, Depue RH and Judd HL (1988) The early in utero oestrogen and testosterone environment of blacks and whites: potential effects on male offspring. Br J Cancer 57,216–218.
Henderson JK, Draper JS, Baillie HS, Fishel S, Thomson JA, Moore H and Andrews PW (2002) Preimplantation human embryos and embryonic stem cells show comparable expression of stage-specific embryonic antigens. Stem Cells 20,329–337.
Herbst H, Sauter M and Mueller-Lantzsch N (1996) Expression of human endogeneous retrovirus K elements in germ cell and trophoblastic tumours. Am J Pathol 149,1727–1735.
Higuchi TT, Palmer JS, Gray LE Jr and Veeramachaneni DN (2003) Effects of dibutyl phthalate in male rabbits following in utero, adolescent, or postpubertal exposure. Toxicol Sci 72,301–313.
Hoei-Hansen CE, Holm M, Rajpert-De Meyts E and Skakkebæk NE (2003) Histological evidence of testicular dysgenesis in contralateral biopsies of 218 patients with testicular germ cell cancer. J Pathol 200,370–374.
Hoei-Hansen CE, Nielsen JE, Almstrup K, Hansen MA, Skakkebaek NE, Rajpert-De Meyts E and Leffers H (2004a) Identification of genes differentially expressed in testes containing carcinoma in situ. Mol Hum Reprod 10,423–431.
Hoei-Hansen CE, Nielsen JE, Almstrup K, Brask Sonne S, Græm N, Skakkebæk NE, Leffers H and Rajpert-De Meyts E (2004b) Transcription factor AP-2γ is a developmentally regulated marker of testicular carcinoma *in situ* and germ cell tumors. Clin Cancer Res 10,8521–8530.
Hoei-Hansen CE, Rajpert-De Meyts E, Carlsen E, Almstrup K, Leffers H and Skakkebæk NE (2005a) A subfertile patient diagnosed with testicular

carcinoma in situ by immunocytological staining for AP-2γ in semen samples: case report. Hum Reprod 20,579–582.
Hoei-Hansen CE, Almstrup K, Nielsen JE, Brask Sonne S, Graem N, Skakkebæk NE, Leffers H and Rajpert-De Meyts E (2005b) Stem cell pluripotency factor NANOG is expressed in human fetal gonocytes, testicular carcinoma *in situ* and germ cell tumours. Histopathology 47,48–56.
Höfken K and Lauke H (1996) Mitotic frequency in different early stages of testicular seminoma. Andrologia 28,335–341.
Holm M, Lenz S, Rajpert-De Meyts E and Skakkebæk NE (2001) Microcalcifications and carcinoma in situ of the testis. BJU Int 87,1–8.
Holm M, Hoei-Hansen CE, Rajpert-De Meyts E and Skakkebæk NE (2003) Increased risk of carcinoma in situ in patients with testicular germ cell cancer with ultrasonic microlithiasis in the contralateral testicle. J Urol 170,1163–1167.
Holstein AF and Körner F (1974) Light and electron microscopical analysis of cell types in human seminoma. Virchows Arch A Pathol Anat Histol 363,97–112.
Honecker F, Stoop H, de Krijger RR, Lau Y-FC, Bokemeyer C and Looijenga LH (2004) Pathobiological implications of the expression of markers of testicular carcinoma in situ by fetal germ cells. J Pathol 203,849–857.
Honorio S, Agathanggelou A, Wernert N, Rothe M, Maher ER and Latif F (2003) Frequent epigenetic inactivation of the RASSF1A tumour suppressor gene in testicular tumours and distinct methylation profiles of seminoma and nonseminoma testicular germ cell tumours. Oncogene 22,461–466.
Houldsworth J, Reuter C, Bosl GJ and Chaganti RSK (1997) Aberrant expression of cyclin D2 is an early event in human male germ cell tumorigenesis. Cell Growth Differ 8,293–299.
Huang E, Nocka K, Beier DR, Chu TY, Buck J, Lahm HW, Wellner D, Leder P and Besmer P (1990) The hematopoietic growth factor KL is encoded by the Sl locus and is the ligand of the c-kit receptor, the gene product of the W locus. Cell 63,225–233.
Hucklenbroich K, Gromoll J, Heinrich M, Hohoff C, Nieschlag E and Simoni M (2005) Partial deletions in the AZFc region of the Y chromosome occur in men with impaired as well as normal spermatogenesis. Hum Reprod 20,191–197.
Huff DS, Hadziselimovic F, Snyder HM, Blythe B and Duckett JW (1993) Histologic maldevelopment of unilaterally cryptorchid testis and their descended partners. Eur J Pediatr 152,S11–S14.
Hughes IA, Lim HN, Martin H, Mongan NP, Dovey L, Ahmed SF and Hawkins JR (2001) Developmental aspects of androgen action. Mol Cell Endocrinol 185,33–41.
Hustin J, Collette J and Franchimont P (1987) Immunohistochemical demonstration of placental alkaline phosphatase in various states of testicular development and in germ cell tumours. Int J Androl 10,29–35.
Hutson JM, Hasthorpe S and Heyns CF (1997) Anatomical and functional aspects of testicular descent and cryptorchidism. Endocr Rev 18,259–280.
Huyghe E, Matsuda T and Thonneau P (2003) Increasing incidence of testicular cancer worldwide: a review. J Urol 170,5–11.
Irvine S, Cawood E, Richardson D, MacDonald E and Aitken J (1996) Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. BMJ 312,467–471.
Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR and Coetzee GA (2000) Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum Mol Genet 9,267–274.
Ishii T, Sato S, Kosaki K, Sasaki G, Muroya K, Ogata T and Matsuo N (2001) Micropenis and the AR gene: mutation and CAG repeat-length analysis. J Clin Endocrinol Metab 86,5372–5378.
Ivell R and Bathgate RA (2002) Reproductive biology of the relaxin-like factor (RLF/INSL3). Biol Reprod 67,699–705.
Ivell R and Hartung S (2003) The molecular basis of cryptorchidism. Mol Hum Reprod 9,175–181.
Izquierdo MA, Van der Valk P, Van Ark-Otte J, Rubio G, Germa-Lluch JR, Ueda R, Scheper RJ, Takahashi T and Giaccone G (1995) Differential expression of the c-kit proto-oncogene in germ cell tumours. J Pathol 177,253–258.
Jacobsen GK and Nørgaard-Pedersen B (1984) Placental alkaline phosphatase in testicular germ cell tumours and in carcinoma-in-situ of the testis. Acta Pathol Microbiol Immunol Scand [A] 92,323–929.
Jacobsen GK and Henriques UV (1992) A fetal testis with intratubular germ cell neoplasia (ITGCN). Mod Pathol 5,547–549.
Jacobsen R, Bostofte E, Engholm G, Hansen J, Skakkebaek NE and Møller H (2000a) Fertility and offspring sex ratio of men who develop testicular cancer: a record linkage study. Hum Reprod 15,1958–1961.
Jacobsen R, Bostofte E, Engholm G, Hansen J, Olsen JH, Skakkebaek NE and Møller H (2000b) Risk of testicular cancer in men with abnormal semen characteristics: cohort study. BMJ 321,789–792.
Jensen TK, Vierula M, Hjollund NH, Saaranen M, Scheike T, Saarikoski S, Suominen J, Keiski A, Toppari J and Skakkebæk NE (2000) Semen quality among Danish and Finnish men attempting to conceive. The Danish First Pregnancy Planner Study Team. Eur J Endocrinol 142,47–52.
Jensen TK, Jorgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, Carlsen E, Magnus O et al. (2004) Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770 young men from the general population in five European countries. Am J Epidemiol 159,49–58.
Jones TD, Ulbright TM, Eble JN and Cheng L (2004) OCT4: a sensitive and specific biomarker for intratubular germ cell neoplasia of the testis. Clin Cancer Res 10,8544–8547.
Jørgensen N, Giwercman A, Müller J and Skakkebæk NE (1993) Immunohistochemical markers of carcinoma in situ of the testis also expressed in normal infantile germ cells. Histopathology 22,373–378.
Jørgensen N, Muller J, Giwercman A, Visfeldt J, Moller H and Skakkebæk NE (1995a) DNA content and expression of tumour markers in germ cells adjacent to germ cell tumours in childhood: probably a different origin for infantile and adolescent germ cell tumours. J Pathol 176,269–278.
Jørgensen N, Rajpert-De Meyts E, Graem N, Müller J, Giwercman A and Skakkebæk NE (1995b) Expression of immunohistochemical markers for testicular carcinoma in situ by normal human fetal germ cells. Lab Invest 72,223–231.
Jørgensen N, Müller J, Jaubert F, Clausen OP and Skakkebæk NE (1997) Heterogeneity of gonadoblastoma germ cells: similarities with immature germ cells, spermatogonia and testicular carcinoma in situ cells. Histopathology 30,177–186.
Jørgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, Andersen AN, Auger J, Cawood EHH, Horte A et al. (2001) Regional differences in semen quality in Europe. Hum Reprod 16,1012–1019.
Jørgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen AG, Andersson AM, Haugen TB, Horte A, Jensen TK et al. (2002) East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum Reprod 17,2199–2208.
Jungbluth AA, Busam KJ, Kolb D, Iversen K, Coplan K, Chen YT, Spagnoli GC and Old LJ (2000) Expression of MAGE-antigens in normal tissues and cancer. Int J Cancer 85,460–465.
Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F and Pinkel D (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258,818–821.
Kamp C, Hirschmann P, Voss H, Huellen K and Vogt PH (2000) Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. Hum Mol Genet 9,2563–2572.
Kang J-L, Rajpert-De Meyts E, Wiels J and Skakkebæk NE (1995) Expression of the glycolipid globotriaosylceramide (Gb3) in testicular carcinoma in situ. Virchows Arch 426,369–374.
Kang J-L, Rajpert-De Meyts E and Skakkebæk NE (1996) Immunoreactive neuron-specific enolase (NSE) is expressed in testicular carcinoma-in-situ. J Pathol 178,161–165.
Kawakami T, Okamoto K, Sugihara H, Hattori T, Reeve AE, Ogawa O and Okada Y (2003) The roles of supernumerical X chromosomes and XIST expression in testicular germ cell tumors. J Urol 169,1546–1552.
Kemmer K, Corless CL, Fletcher JA, McGreevey L, Haley A, Griffith D, Cummings OW, Wait C, Town A and Heinrich MC (2004) KIT mutations are common in testicular seminomas. Am J Pathol 164,305–313.
Kersemaekers A-MF, Honecker F, Stoop H, Cools M, Molier M, Wolffenbutel K, Bokemeyer C, Li Y, Lau Y-FC, Oosterhuis JW et al. (2005) Identification of germ cells at risk of neoplastic transformation in gonadoblastoma. An immunohistochemical study of OCT3/4 and TSPY. Hum Pathol 36,512–521.
Klein EA, Chen RN, Levin HS, Rackley RR and Williams BR (1996) Testicular cancer in association with developmental renal anomalies and hypospadias. Urology 47,82–87.
Klonisch T, Fowler PA and Hombach-Klonisch S (2004) Molecular and genetic regulation of testis descent and external genitalia development. Dev Biol 270,1–18.
Kolon TF, Patel RP and Huff DS (2004) Cryptorchidism: diagnosis, treatment, and long-term prognosis. Urol Clin North Am 31,469–480.
Koul S, Houldsworth J, Mansukhani MM, Donadio A, McKiernan JM, Reuter VE, Bosl GJ, Chaganti RS and Murty VV (2002) Characteristic promoter hypermethylation signatures in male germ cell tumors. Mol Cancer 1,8.
Krabbe S, Skakkebæk NE, Berthelsen JG, Eyben FV, Volsted P, Mauritzen K, Eldrup J and Nielsen AH (1979) High incidence of undetected neoplasia in maldescended testes. Lancet 1,999–1000.

Kraggerud SM, Berner A, Bryne M, Pettersen EO and Fosså SD (1999) Spermatocytic seminoma as compared to classical seminoma: an immunohistochemical and DNA flow cytometric study. APMIS 107,297–302.

Kraggerud SM, Skotheim RI, Szymanska J, Eknaes M, Fossa SD, Stenwig AE, Peltomaki P and Lothe RA (2002) Genome profiles of familial/bilateral and sporadic testicular germ cell tumors. Genes Chromosomes Cancer 34,168–174.

Krausz CG, Quintana-Murci L and McElreavey K (2000) Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Hum Reprod 15,1431–1434.

Krausz CG, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K and Skakkebæk NE (2001a) Double blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles. J Clin Endocrinol Metab 86,2638–2642.

Krausz CG, Quintana-Murci L, Rajpert-De Meyts E, Jørgensen N, Jobling M, Rosser ZH, Skakkebæk NE and McElreavey K (2001b) Identification of a Y chromosome haplogroup associated with reduced sperm counts. Hum Mol Genet 10,1873–1877.

Krausz C, Quintana-Murci L and Forti G (2004) Y chromosome polymorphisms in medicine. Ann Med 36,573–583.

Kula K (1988) Induction of precocious maturation of spermatogenesis in infant rats by human menopausal gonadotropin and inhibition by simultaneous administration of gonadotropins and testosterone. Endocrinology 122,34–39.

Kumagai J, Hsu SY, Roh JS, Fu P, Wade JD, Bathgate RA and Hsueh AJ (2002) INSL3/Leydig insulin-like peptide activates the LGR8 receptor important in testis descent. J Biol Chem 277,31283–31286.

Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S et al. (2001) The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet 29,279–286.

Kuroki Y, Iwamoto T, Lee J, Yoshiike M, Nozawa S, Nishida T, Ewis AA, Nakamura H, Toda T, Tokunaga K et al. (1999) Spermatogenic ability is different among males in different Y chromosome lineage. J Hum Genet 44,289–292.

La Spada AR, Wilson EM, Lubahn DB, Harding AE and Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352,77–79.

Lahn BT and Page DC (1997) Functional coherence of the Y chromosome. Science 278,675–680.

Laml T, Preyer O, Umek W, Hengstschlager M and Hanzal H (2002) Genetic disorders in premature ovarian failure. Hum Reprod Update 8,483–441.

Lau Y-FC, Chou PM, Iezzoni JC, Alonzo JA and Kömüves LG (2000) Expression of a candidate gene for the gonadoblastoma locus in gonadoblastoma and testicular seminoma. Cytogenet Cell Genet 91,160–164.

Liao C, Li SQ, Wang X, Muhlrad S, Bjartell A and Wolgemuth DJ (2004) Elevated levels and distinct patterns of expression of A-type cyclins and their associated cyclin-dependent kinases in male germ cell tumors. Int J Cancer 108,654–664.

Lifschitz-Mercer B, Elliott DJ, Leider-Trejo L, Schreiber-Bramante L, Hassner A, Eisenthal A and Maymon BB (2000) Absence of RBM expression as a marker of intratubular (in situ) germ cell neoplasia of the testis. Hum Pathol 31,1116–1120.

Lifschitz-Mercer B, Elliott DJ, Issakov J, Leider-Trejo L, Schreiber L, Misonzhnik F, Eisenthal A and Maymon BB (2002) Localization of a specific germ cell marker, DAZL1, in testicular germ cell neoplasias. Virchows Arch 440,387–391.

Lim HN, Chen H, McBride S, Dunning AM, Nixon RM, Hughes IA and Hawkins JR (2000) Longer polyglutamine tracts in the androgen receptor are associated with moderate to severe undermasculinized genitalia in XY males. Hum Mol Genet 22,829–834.

Lim HN, Nixon RM, Chen H, Hughes IA and Hawkins JR (2001) Evidence that longer androgen receptor polyglutamine repeats are a causal factor for genital abnormalities. J Clin Endocrinol Metab 86,3207–3210.

Looijenga LHJ, Gillis AJM, van Gurp RJHM, Verkerk AJMH and Oosterhuis JW (1997) X inactivation in human testicular tumours. XIST expression and androgen receptor methylation status. Am J Pathol 151,581–590.

Looijenga LHJ, De Leeuw PJC, Van Oorschot M, van Gurp RJH, Stoop H, Gillis AJ, de Gouveia Brazao CA, Weber RF, Kirkels WJ, van Dijk T et al. (2003a) Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ cell tumors. Cancer Res 63,7674–7678.

Looijenga LHJ, Stoop H, De Leeuw PJC, De Gouveia Brazao CA, Gillis AJM, van Roozendaal KE, van Zoelen EJ, Weber RF, Wolffenbuttel KP, van Dekken H et al. (2003b) POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 63,2244–2250.

Looijenga LH, Zafarana G, Grygalewicz B, Summersgill B, Debiec-Rychter M, Veltman J, Schoenmakers EF, Rodriguez S, Jafer O, Clark J et al. (2003c) Role of gain of 12p in germ cell tumour development. APMIS 111,161–171.

Looijenga LH, Hersmus R, Gillis AJ, Pfundt R, Stoop HJ, van Gurp RJ, Veltman J, Beverloo HB, van Drunen E, van Kessel AG, et al. (2006) Genomic and expression profiling of human spermatocytic seminomas: primary spermatocyte as tumorigenic precursor and DMRT1 as candidate chromosome 9 gene. Cancer Res 66,290–302.

Loy V and Dieckmann K-P (1990) Carcinoma in situ of the testis: Intratubular germ cell neoplasia or testicular intraepithelial neoplasia? Hum Pathol 21,457–458.

Luetjens CM, Gromoll J, Engelhardt M, Von Eckardstein S, Bergmann M, Nieschlag E and Simoni M (2002) Manifestation of Y-chromosomal deletions in the human testis: a morphometrical and immunohistochemical evaluation. Hum Reprod 17,2258–2266.

Lutke Holzik MF, Rapley EA, Hoekstra HJ, Sleijfer DT, Nolte IM and Sijmons RH (2004) Genetic predisposition to testicular germ cell tumours. Lancet Oncol 5,363–371.

Lutke Holzik MF, Storm K, Sijmons RH, D'hollander M, Arts EG, Verstraaten ML, Sleijfer DT and Hoekstra HJ (2005) Absence of constitutional Y chromosome AZF deletions in patients with testicular germ cell tumors. Urology 65,196–201.

Lynch M, Cram DS, Reilly A, O'Bryan MK, Baker HW, de Kretser DM and McLachlan RI (2005) The Y chromosome gr/gr subdeletion is associated with male infertility. Mol Hum Reprod 11,507–512.

Machev N, Saut N, Longepied G, Terriou P, Navarro A, Levy N, Guichaoua M, Metzler-Guillemain C, Collignon P, Frances AM et al. (2004) Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. J Med Genet 41,814–825. Erratum in J Med Genet (2004) 41,960.

MacMahon RA and Cussen LJ (1991) Detection of gonadal carcinoma in situ in childhood and implications for management. Aust N Z J Surg 61,667–669.

Madani A, Kemmer K, Sweeney C, Corless C, Ulbright T, Heinrich M and Einhorn L (2003) Expression of KIT and epidermal growth factor receptor in chemotherapy refractory non-seminomatous germ-cell tumors. Ann Oncol 14,873–880.

Maio M, Coral S, Fratta E, Altomonte M and Sigalotti L (2003) Epigenetic targets for immune intervention in human malignancies. Oncogene 22,6484–6488.

Maitra A, Arking DE, Shivapurkar N, Ikeda M, Stastny V, Kassauei K, Sui G, Cutler DJ, Liu Y, Brimble SN et al. (2005) Genomic alterations in cultured human embryonic stem cells. Nat Genet 37,1099–1103.

Manuel M, Katayama PK and Jones HW Jr (1976) The age of occurrence of gonadal tumors in intersex patients with a Y chromosome. Am J Obstet Gynecol 124,293–300.

Mark GJ and Hedinger C (1965) Changes in the remaining tumor-free testicular tissue in cases of seminoma and teratoma. Virchows Arch Pathol Anat Physiol Klin Med 340,84–92.

Marks A, Sutherland DR, Bailey D, Iglesias J, Law J, Lei M, Yeger H, Banerjee D and Baumal R (1999) Characterization and distribution of an onco-fetal antigen (M2A antigen) expressed on testicular germ cell tumours. Br J Cancer 80,569–578.

Masters JR and Koberle B (2003) Curing metastatic cancer: lessons from testicular germ-cell tumours. Nat Rev Cancer 3,517–525.

McElreavey K and Quintana-Murci L (2003) Y chromosome haplogroups: a correlation with testicular dysgenesis syndrome? APMIS 111,106–114.

Mifsud A, Sim CK, Boettger-Tong H, Moreira S, Lamb DJ, Lipshultz LI and Yong EL (2001) Trinucleotide (CAG) repeat polymorphism in the androgen receptor gene: molecular markers of risk for male infertility. Fertil Steril 75,275–281.

Mihara K, Cao XR, Yen A, Chandler S, Driscoll B, Murphree AL, T'Ang A and Fung. YK (1989) Cell cycle-dependent regulation of phosphorylation of the human retinoblastoma gene product. Science 246,1300–1303.

Milatiner D, Halle D, Huerta M, Margalioth EJ, Cohen Y, Ben-Chetrit A, Gal M, Mimoni T and Eldar-Geva T (2004) Associations between androgen receptor CAG repeat length and sperm morphology. Hum Reprod 19,1426–1430.

Møller H (1993) Clues to aetiology of testicular germ cell tumours from descriptive epidemiology. Eur Urol 23,8–15.

Møller H and Skakkebaek NE (1999) Risk of testicular cancer in subfertile men: case-control study. BMJ 318,559–562.

Morley R and Lucas A (1987) Undescended testis in low birth weight infants. BMJ 295,753.

Morrison AS (1976) Cryptorchidism, hernia, and cancer of the testis. J Natl Cancer Inst 56,731–733.
Müller J and Skakkebæk NE (1981) Microspectrophotometric DNA measurements of carcinoma-in-situ germ cells in the testis. Int J Androl 4,211–221.
Müller J and Skakkebæk NE (1984) Testicular carcinoma in situ in children with androgen insensitivity (testicular feminisation) syndrome. BMJ 288,1419–1420.
Müller J, Skakkebæk NE, Ritzén EM, Plöen L and Petersen KE (1985) Carcinoma in situ of the testis in children with 45,X/46,XY gonadal dysgenesis. J Pediatr 106,431–436.
Müller J, Skakkebæk NE and Parkinson MC (1987) The spermatocytic seminoma: views on pathogenesis. Int J Androl 10,147–156.
Muroya K, Sasagawa I, Suzuki Y, Nakada T, Ishii T and Ogata T (2001) Hypospadias and the androgen receptor gene: mutation screening and CAG repeat length analysis. Mol Hum Reprod 7,409–413.
Nathanson KL, Kanetsky PA, Hawes R, Vaughn DJ, Letrero R, Tucker K, Friedlander M, Phillips KA, Hogg D, Jewett MA, et al. (2005) The Y deletion gr/gr and susceptibility to testicular germ cell tumor. Am J Hum Genet 77,1034–1043.
Nef S and Parada LF (1999) Cryptorchidism in mice mutant for *Insl3*. Nat Genet 22,295–299.
Nielsen H, Nielsen M and Skakkebæk NE (1974) The fine structure of a possible carcinoma-in-situ in the seminiferous tubules in the testis of four infertile men. APMIS 82,235–248.
Ochsenkühn R and de Kretser DM (2003) The contributions of deficient androgen action in spermatogenic disorders. Int J Androl 26,195–201.
Okada K, Katagiri T, Tsunoda T, Mizutani Y, Suzuki Y, Kamada M, Fujioka T, Shuin T, Miki T and Nakamura Y (2003) Analysis of gene-expression profiles in testicular seminomas using a genome-wide cDNA microarray. Int J Oncol 23,1615–1635.
Oosterhuis JW and Looijenga LHJ (2005) Testicular germ-cell tumours in a broader perspective. Nat Rev Cancer 5,210–222.
Oosterhuis JW, Castedo SM, de Jong B, Cornelisse CJ, Dam A, Sleijfer DT and Schraffordt Koops H (1989) Ploidy of primary germ cell tumors of the testis. Pathogenetic and clinical relevance. Lab Invest 60,14–21.
Oosterhuis JW, Castedo SM and de Jong B (1990) Cytogenetics, ploidy and differentiation of human testicular, ovarian and extragonadal germ cell tumours. Cancer Surv 9,320–332.
Ottesen AM, Skakkebæk NE, Lundsteen C, Leffers H, Larsen J and Rajpert-De Meyts E (2003) High resolution comparative genomic hybridisation detects extra chromosome 12p material in most cases of carcinoma in situ (CIS) adjacent to overt germ cell tumours, but not in CIS prior to the tumour development. Genes Chromosomes Cancer 38,117–125.
Ottesen AM, Larsen J, Skakkebæk NE, Gerdes T, Christensen IJ, Larsen JK, Lundsteen C and Rajpert-De Meyts E (2004a) Cytogenetic investigation of testicular carcinoma in situ and early seminoma by high resolution comparative genomic hybridisation on subpopulations with specific DNA content. Cancer Genet Cytogenet 149,89–97.
Ottesen AM, Rajpert-De Meyts E, Holm M, Andersen I-LF, Vogt PH, Lundsteen C and Skakkebæk NE (2004b) Cytogenetic and molecular analysis of a family with three brothers afflicted with germ cell cancer. Clin Genet 65,32–39.
Palumbo C, van Roozendaal K, Gillis AJ, van Gurp RH, de Munnik H, Oosterhuis JW, van Zoelen EJ and Looijenga LH (2002) Expression of the *PDGF* α-receptor 1.5 kb transcript, *OCT-4*, and c-*KIT* in human normal and malignant tissues. Implications for the early diagnosis of testicular germ cell tumours and for our understanding of regulatory mechanisms. J Pathol 196,467–477.
Park SP, Lee YJ, Lee KS, Ah Shin H, Cho HY, Chung KS, Kim EY and Lim JH (2004) Establishment of human embryonic stem cell lines from frozen-thawed blastocysts using STO cell feeder layers. Hum Reprod 19,676–684.
Parkinson MC and Harland SJ (2002) Expert commentary. Histopathology 41,2–4.
Paulozzi LJ (1999) International trends in rates of hypospadias and cryptorchidism. Environ Health Perspect 107,297–302.
Pauls K, Jager R, Weber S, Wardelmann E, Koch A, Buttner R and Schorle H (2005) Transcription factor AP-2 gamma, a novel marker of gonocytes and seminomatous germ cell tumours. Int J Cancer 115,470–477.
Peltomäki P, Lothe R, Borresen AL, Fosså SD, Brogger A and de la Chapelle A (1991) Altered dosage of the sex chromosomes in human testicular cancer: a molecular genetic study. Int J Cancer 47,518–522.
Perlman EJ, Hu J, Ho D, Cushing B, Lauer S and Castleberry RP (2000) Genetic analysis of childhood endodermal sinus tumors by comparative genomic hybridisation. J Pediatr Hematol Oncol 22,100–105.
Pettersson A, Kaijser M, Richiardi L, Askling J, Ekbom A and Akre O (2004) Women smoking and testicular cancer: one epidemic causing another? Int J Cancer 109,941–944.
Prener A, Engholm G and Jensen OM (1996) Genital anomalies and risk for testicular cancer in Danish men. Epidemiology 7,14–19.
Pugh RCB and Parkinson C (1981) The origin and classification of testicular germ cell tumours. Int J Androl Suppl.4,15–25.
Quenby SM, Gazvani MR, Brazeau C, Neilson J, Lewis-Jones DI and Vince G (1999) Oncogenes and tumour suppressor genes in first trimester human fetal gonadal development. Mol Hum Reprod 5,737–741.
Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM and French FS (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16,271–321.
Quintana-Murci L, Weale ME, Thomas MG, Erdei E, Bradman N, Shanks JH, Krausz CG and McElreavey K (2003) Y-chromosome haplotypes and testicular cancer in the English population. J Med Genet 40,E20.
Rajpert-De Meyts E and Skakkebæk NE (1993) The possible role of sex hormones in the development of testicular cancer. Eur Urol 23,54–59.
Rajpert-De Meyts E and Skakkebæk NE (1994) Expression of the c-kit protein product in carcinoma in situ and invasive germ cell tumours. Int J Androl 17,85–92.
Rajpert-De Meyts E, Jørgensen N, Müller J and Skakkebæk NE (1996a) Prolonged expression of the c-kit receptor in germ cells of intersex fetal testes. J Pathol 178,166–169.
Rajpert-De Meyts E, Kvist M and Skakkebæk NE (1996b) Heterogeneity of expression of immunohistochemical tumour markers in the testicular carcinoma-in situ: pathogenetic relevance. Virchows Arch 428,133–139.
Rajpert-De Meyts E, Jørgensen N, Brøndum-Nielsen K, Müller J and Skakkebæk NE (1998a) Developmental arrest of germ cells in the pathogenesis of germ cell neoplasia. APMIS 106,198–206.
Rajpert-De Meyts E, Lauke H and Skakkebæk NE (1998b) In vitro survival of human neoplastic germ cells. Adv Exp Med Biol 444,59–66.
Rajpert-De Meyts E, Jørgensen NE, Græm N, Müller J, Cate R and Skakkebæk NE (1999) Expression of anti-Müllerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab 84,3836–3844.
Rajpert-De Meyts E, Leffers H, Petersen HJ, Andersen A-G, Carlsen E, Jørgensen N and Skakkebæk NE (2002a) The CAG repeat length in the androgen receptor gene and reproductive variables in fertile and infertile men. Lancet 359,44–46.
Rajpert-De Meyts E, Leffers H, Daugaard G, Andersen CB, Petersen PM, Hinrichsen J, Pedersen LG and Skakkebæk NE (2002b) Analysis of the polymorphic CAG repeat length in the androgen receptor gene in patients with testicular germ cell cancer. Int J Cancer 102,201–204.
Rajpert-De Meyts E, Bartkova J, Samson M, Hoei-Hansen CE, Frydelund-Larsen L, Bartek J and Skakkebæk NE (2003a) The emerging phenotype of the testicular carcinoma in situ germ cell. APMIS 111,267–279.
Rajpert-De Meyts E, Jacobsen GK, Bartkova J, Aubry F, Samson M, Bartek J and Skakkebæk NE (2003b) The immunohistochemical expression pattern of Chk2, 19-INK4d, 53, MAGE–A4 and other selected antigens provides new evidence for the pre-meiotic origin of spermatocytic seminoma. Histopathology 42,1–10.
Rajpert-De Meyts E, Hanstein R, Jørgensen N, Græm N, Vogt PH and Skakkebæk NE (2004) Developmental expression of the *POU5F1* (OCT-3/4) in normal and dysgenetic human gonads. Hum Reprod 19,1338–1344.
Ramani P, Yeung CK and Habeebu SSM (1993) Testicular intratubular germ cell neoplasia in children and adolescents with intersex. Am J Surg Pathol 17,1124–1133.
Rapley EA, Crockford GP, Teare D, Biggs P, Seal S, Barfoot R, Edwards S, Hamoudi R, Heimdal K, Fosså SD et al. (2000) Localization to Xq27 of a susceptibility gene for testicular germ-cell tumours. Nat Genet 24,197–200.
Rapley EA, Hockley S, Warren W, Johnson L, Huddart R, Crockford G, Forman D, Leahy MG, Oliver DT, Tucker K et al. (2004) Somatic mutations of KIT in familial testicular germ cell tumours. Br J Cancer 90,2397–2401.
Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, 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 RA, Dorfman DM, Slee R, Renshaw AA, Loughlin KR, Cooke H and Page DC (2000) DAZ family proteins exist throughout male germ cell development and transit from nucleus to cytoplasm at meiosis in humans and mice. Biol Reprod 63,1490–1496.
Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, Page DC and Rozen S (2002) Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet 71,906–922.
Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, Kuroda-Kawaguchi T, de Vries JW, Oates RD, Silber S et al. (2003)

Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet 35,247–251.

Repping S, van Daalen SK, Korver CM, Brown L, Marszalek J, Gianotten J, Oates RD, Silber S, van der Veen F, Page DC et al. (2004) A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the AZFc region. Genomics 83,1046–1052.

Rey R, al-Attar L, Louis F, Jaubert F, Barbet P, Nihoul-Fekete C, Chaussain JL and Josso N (1996) Testicular dysgenesis does not affect expression of anti-Mullerian hormone by Sertoli cells in premeiotic seminiferous tubules. Am J Pathol 148,1689–1698.

Richard SM, Bianchi NO, Bianchi MS, Peltomaki P, Lothe RA and Pavicic W (2004) Ethnic variation in the prevalence of AZF deletions in testicular cancer. Mutat Res 554,45–51.

Richiardi L, Bellocco R, Adami HO, Torrang A, Barlow L, Hakulinen T, Rahu M, Stengrevics A, Storm H, Tretli S et al. (2004a) Testicular cancer incidence in eight northern European countries: secular and recent trends. Cancer Epidemiol Biomarkers Prev 13,2157–2166.

Richiardi L, Akre O, Lambe M, Granath F, Montgomery SM and Ekbom A (2004b) Birth order, sibship size, and risk for germ-cell testicular cancer. Epidemiology 15,323–329.

Richiardi L, Akre O, Montgomery SM, Lambe M, Kvist U and Ekbom A (2004c) Fecundity and twinning rates as measures of fertility before diagnosis of germ-cell testicular cancer. J Natl Cancer Inst 96,145–147.

Robinson LL, Gaskell TL, Saunders PT and Anderson RA (2001) Germ cell specific expression of c-kit in the human fetal gonads. Mol Hum Reprod 7,845–852.

Rodriguez E, Mathew S, Reuter V, Ilson DH, Bosl GJ and Chaganti RS (1992) Cytogenetic analysis of 124 prospectively ascertained male germ cell tumors. Cancer Res 52,2285–2291.

Rodriguez E, Houldsworth J, Reuter VE, Meltzer P, Zhang J, Trent JM, Bosl GJ and Chaganti RS (1993) Molecular cytogenetic analysis of i(12p)-negative human male germ cell tumors. Genes Chromosomes Cancer 8,230–236.

Rodriguez S, Jafer O, Goker H, Summersgill BM, Zafarana G, Gillis AJ, van Gurp RJ, Oosterhuis JW, Lu YJ, Huddart R et al. (2003) Expression profile of genes from 12p in testicular germ cell tumors of adolescents and adults associated with i(12p) and amplification at 12p11.2-p12 1. Oncogene 22,1880–1891.

Roelofs H, van Gurp RJ, Oosterhuis JW and Looijenga LH (1998) Detection of human endogenous retrovirus type K-specific transcripts in testicular parenchyma and testicular germ cell tumors of adolescents and adults: clinical and biological implications. Am J Pathol 153,1277–1282.

Roelofs H, Mostert MC, Pompe K, Zafarana G, van Oorschot M, van Gurp RJ, Gillis AJ, Stoop H, Beverloo B, Oosterhuis JW et al. (2000) Restricted 12p amplification and RAS mutation in human germ cell tumors of the adult testis. Am J Pathol 157,1155–1166.

Rørth M, Rajpert-De Meyts E, Andersson L, Dieckmann K-P, Fosså SD, Grigor KM, Hendry WH, Herr HW, Looijenga LHJ, Oosterhuis JW et al. (2000) Carcinoma in situ in the testis. Scand J Urol Nephrol 34,166–186.

Rosenberg C, Van Gurp RJ, Geelen E, Oosterhuis JW and Looijenga LH (2000) Overrepresentation of the short arm of chromosome 12 is related to invasive growth of human testicular seminomas and nonseminomas. Oncogene 19,5858–5862.

Ross RK, Pike MC, Coetzee GA, Reichardt JK, Yu MC, Feigelson H, Stanczyk FZ, Kolonel LN and Henderson BE (1998) Androgen metabolism and prostate cancer: establishing a model of genetic susceptibility. Cancer Res 58,4497–4504.

Rutgers JL and Scully RE (1991) The androgen insensitivity syndrome (testicular feminization): a clinicopathologic study of 43 cases. Int J Gynecol Pathol 10,126–144.

Salo P, Kaariainen H, Petrovic V, Peltomaki P, Page DC and de la Chapelle A (1995) Molecular mapping of the putative gonadoblastoma locus on the Y chromosome. Genes Chromosomes Cancer 14,210–214.

Sartor O, Zheng Q and Eastham JA (1999) Androgen receptor gene CAG repeat length varies in a race-specific fashion in men without prostate cancer. Urology 53,378–380.

Sasagawa I, Suzuki Y, Tateno T, Nakada T, Muroya K and Ogata T (2000) CAG repeat length of the androgen receptor gene in Japanese males with cryptorchidism. Mol Hum Reprod 6,973–975.

Satie A-P, Rajpert-De Meyts E, Spagnoli GC, Henno S, Olivo L, Jacobsen GK, Rioux-Leclercq N, Jegou B and Samson M (2002) The cancer-testis gene, *NY-ESO-1* is expressed in normal foetal and adult testes, in spermatocytic seminoma and testicular carcinoma in situ. Lab Invest 82, 775–780.

Sato N, Sanjuan IM, Heke M, Uchida M, Naef F and Brivanlou AH (2003) Molecular signature of human embryonic stem cells and its comparison with the mouse. Dev Biol 260,404–413.

Savage MO and Lowe DG (1990) Gonadal neoplasia and abnormal sexual differentiation. Clin Endocrinol (Oxf) 32,519–533.

Scanlan MJ, Gure AO, Jungbluth AA, Old LJ and Chen YT (2002) Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. Immunol Rev 188,22–32.

Schlessinger D, Herrera L, Crisponi L, Mumm S, Percesepe A, Pellegrini M, Pilia G and Forabosco A (2002) Genes and translocations involved in POF. Am J Med Genet 111,328–333.

Schmidt BA, Rose A, Steinhoff C, Strohmeyer T, Hartmann M and Ackermann R (2001) Up-regulation of cyclin-dependent kinase 4/cyclin D2 expression but down-regulation of cyclin-dependent kinase 2/cyclin E in testicular germ cell tumors. Cancer Res 61,4214–4221.

Schneider DT, Schuster AE, Fritsch MK, Hu J, Olson T, Lauer S, Göbel U, Perlman E and Pediatric Oncology Group and German Pediatric Germ Cell Tumor Study Group (2001) Multipoint imprinting analysis indicates a common precursor cell for gonadal and nongonadal pediatric germ cell tumors. Cancer Res 61,7268–7276.

Schnieders F, Dörk T, Arnemann J, Vogel T, Werner M and Schmidtke J (1996) Testis-specific protein, Y-encoded (TSPY) expression in testicular tissues. Hum Mol Genet 5,1801–1807.

Schöler HR, Hatzopoulos AK, Balling R, Suzuki N and Gruss P (1989) A family of octamer-specific proteins present during mouse embryogenesis: evidence for germline-specific expression of an Oct factor. EMBO J 8,2543–2550.

Schreiber L, Lifschitz-Mercer B, Paz G, Yavetz H, Elliott DJ, Kula K, Slowikowska-Hilczer J and Maymon BB (2003) Double immunolabeling by the RBM and the PLAP markers for identifying intratubular (in situ) germ cell neoplasia of the testis. Int J Surg Pathol 11,17–20.

Scully RE (1970) Gonadoblastoma. A review of 74 cases. Cancer 25,1340–1356.

Scully RE (1981) Neoplasia associated with anomalous sexual development and abnormal sex chromosomes. Ped Adolesc Endocrinol 8,203–217.

Sharpe RM and Skakkebaek NE (1993) Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341,1392–1395.

Sharpe RM and Franks S (2002) Environment, lifestyle and infertility? An inter-generational issue. Nat Med 8(Suppl. 2),S33–S40.

Sherr CJ (2004) Principles of tumor suppression. Cell 116,235–246.

Sicinski P, Donaher JL, Geng Y, Parker SB, Gardner H, Park MY, Robker RL, Richards JS, McGinnis LK, Biggers JD et al. (1996) Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. Nature 384,470–474.

Sinke RJ, Suijkerbuijk RF, de Jong B, Oosterhuis JW and Geurts van Kessel A (1993) Uniparental origin of i(12p) in human germ cell tumors. Genes Chromosomes Cancer 6,161–165.

Skakkebæk NE (1972) Possible carcinoma-in-situ of the testis. Lancet ii,516–517.

Skakkebæk NE (1979) Carcinoma-in-situ of testis in testicular feminization syndrome. APMIS 87,87–89.

Skakkebæk NE (1981) Early detection of testicular cancer. An introduction. Int J Androl 4,11–13.

Skakkebæk NE, Berthelsen JG, Giwercman A and Müller J (1987) Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. Int J Androl 10,19–28.

Skakkebæk NE, Rajpert-De Meyts E and Main KM (2001) Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16,972–978.

Skakkebæk NE, Hoei-Hansen CE, Holm M, Jørgensen N and Rajpert-De Meyts E (2003) Association between testicular dysgenesis syndrome (TDS) and testicular neoplasia: evidence from 20 adult patients with signs of maldevelopment of the testis. APMIS 111,1–11.

Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T et al. (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 423,825–837.

Skotheim RI and Lothe RA (2003) The testicular germ cell tumour genome. APMIS 111,136–150.

Skotheim RI, Monni O, Mousses S, Fosså SD, Kallioniemi OP, Lothe RA and Kallioniemi A (2002) New insights into testicular germ cell tumorigenesis from gene expression profiling. Cancer Res 62,2359–2364.

Skotheim RI, Abeler VM, Nesland JM, Fosså SD, Holm R, Wagner U, Florenes VA, Aass N, Kallioniemi OP and Lothe RA (2003) Candidate genes for testicular cancer evaluated by in situ protein expression analyses on tissue microarrays. Neoplasia 5,397–404.

Skotheim RI, Lind GE, Monni O, Nesland JM, Abeler V, Fosså SD, Duale N, Brunborg G, Kallioniemi O, Andrews PW et al. (2005) Differentiation of human embryonal carcinomas *in vitro* and *in vivo* reveals expression profiles relevant to normal development. Cancer Res 65,5588–5598.

Slowikowska-Hilczer J, Szarras-Czapnik M and Kula K (2001) Testicular pathology in 46,XY dysgenetic male pseudohermaphroditism: an approach to pathogenesis of testis cancer. J Androl 22,781–792.

Slowikowska-Hilczer J, Romer TE and Kula K (2003) Neoplastic potential of germ cells in relation to disturbances of gonadal organogenesis and changes in karyotype. J Androl 24,270–278.

Smiraglia DJ, Szymanska J, Kraggerud SM, Lothe RA, Peltomaki P and Plass C (2002) Distinct epigenetic phenotypes in seminomatous and nonseminomatous testicular germ cell tumors. Oncogene 21,3909–3916.

Smith-Sorensen B, Lind GE, Skotheim RI, Fosså SD, Fodstad O, Stenwig AE, Jakobsen KS and Lothe RA (2002) Frequent promoter hypermethylation of the O6-methylguanine-DNA Methyltransferase (MGMT) gene in testicular cancer. Oncogene 21,8878–8884.

Sohval AR (1954) Testicular dysgenesis as an etiologic factor in cryptorchidism. J Urol 72,693–702.

Sohval AR (1956) Testicular dysgenesis in relation to neoplasm of the testicle. J Urol 75,285–291.

Spearow JL, Doemeny P, Sera R, Leffler R and Barkley M (1999) Genetic variation in susceptibility to endocrine disruption by estrogen in mice. Science 285,1259–1261.

Sperger JM, Chen X, Draper JS, Antosiewicz JE, Chon CH, Jones SB, Brooks JD, Andrews PW, Brown PO and Thomson JA (2003) Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. Proc Natl Acad Sci USA 100,13350–13355.

Spierings DC, de Vries EG, Vellenga E and de Jong S (2003) The attractive Achilles heel of germ cell tumours: an inherent sensitivity to apoptosis-inducing stimuli. J Pathol 200,137–148.

Stoop H, van Gurp R, de Krijger R, Geurts van Kessel A, Köberle B, Oosterhuis W and Looijenga L (2001) Reactivity of germ cell maturation stage-specific markers in spermatocytic seminoma: diagnostic and etiological implications. Lab Invest 81,919–928.

Strohmeyer T, Peter S, Hartmann M, Munemitsu S, Ackermann R, Ullrich A and Slamon DJ (1991a) Expression of the hst-1 and c-kit protooncogenes in human testicular germ cell tumors. Cancer Res 51,1811–1816.

Strohmeyer T, Reissmann P, Cordon-Cardo C, Hartmann M, Ackermann R and Slamon D (1991b) Correlation between retinoblastoma gene expression and differentiation in human testicular tumors. Proc Natl Acad Sci USA 88,6662–6666.

Strohmeyer T, Reese D, Press M, Ackerman R, Hartmann M and Slamon D (1995) Expression of the c-kit proto-oncogene and its ligand stem cell factor (SCF) in normal and malignant human testicular tissue. J Urol 153,511–515.

Suijkerbuijk RF, Sinke RJ, Meloni AM, Parrington JM, van Echten J, de Jong B, Oosterhuis JW, Sandberg AA and Geurts van Kessel A (1993) Overrepresentation of chromosome 12p sequences and karyotypic evolution in i(12p)-negative testicular germ-cell tumors revealed by fluorescence in situ hybridization. Cancer Genet Cytogenet 70,85–93.

Sultan C, Paris F, Terouanne B, Balaguer P, Georget V, Poujol N, Jeandel C, Lumbroso S and Nicolas JC (2001) Disorders linked to insufficient androgen action in male children. Hum Reprod Update 7,314–322.

Summersgill B, Osin P, Lu YJ, Huddart R and Shipley J (2001) Chromosomal imbalances associated with carcinoma in situ and associated testicular germ cell tumours of adolescents and adults. Br J Cancer 85,213–220.

Sun C, Skaletsky H, Rozen S, Gromoll J, Nieschlag E, Oates R and Page DC (2000) Deletion of azoospermia factor a (AZFa) region of human Y chromosome caused by recombination between HERV15 proviruses. Hum Mol Genet 9,2291–2296.

Teilum G (1976) Special tumours of ovary and testis and related extragonadal lesions. In Comparative Pathology and Histological Identification, 2nd edn. Munksgaard, Copenhagen.

Tian Q, Frierson HF Jr, Krystal GW and Moskaluk CA (1999) Activating c-kit gene mutations in human germ cell tumors. Am J Pathol 154,1643–1647.

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.

Tilford CA, Kuroda-Kawaguchi T, Skaletsky H, Rozen S, Brown LG, Rosenberg M, McPherson JD, Wylie K, Sekhon M, Kucaba TA et al. (2001) A physical map of the human Y chromosome. Nature 409,943–945.

Toledano MB, Hansell AL, Jarup L, Quinn M, Jick S and Elliott P (2003) Temporal trends in orchidopexy, Great Britain, 1992-1998. Environ Health Perspect 111,129–132.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, Jégou B, Jensen TK, Jouannet P, Keiding N et al. (1996) Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104,741–803.

Toppari J, Kaleva M and Virtanen HE (2001) Trends in the incidence of cryptorchidism and hypospadias, and methodological limitations of registry-based data. Hum Reprod Update 7,282–286.

Tsuchiya K, Reijo R, Page DC and Disteche CM (1995) Gonadoblastoma: molecular definition of the susceptibility region on the Y chromosome. Am J Hum Genet 57,1400–1407.

Turner BC, Zhang J, Gumbs AA, Maher MG, Kaplan L, Carter D, Glazer PM, Hurst HC, Haffty BG and Williams T (1998) Expression of AP-2 transcription factors in human breast cancer correlates with the regulation of multiple growth factor signalling pathways. Cancer Res 58,5466–5472.

Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L and Yong EL (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.

Ulbright TM, Amin MB and Young RH (1999) Atlas of Tumor Pathology. Tumors of the Testis, Adnexa, Spermatic Cord, and Scrotum. Armed Forces Institute of Pathology, Washington DC.

Van Echten J, Oosterhuis JW, Looijenga LH, van de Pol M, Wiersema J, te Meerman GJ, Schaffordt Koops H, Sleijfer DT and de Jong B (1995a) No recurrent structural abnormalities apart from i(12p) in primary germ cell tumors of the adult testis. Genes Chromosomes Cancer 14,133–144.

Van Echten J, Van Gurp RJHLM, Stoepker M, Looijenga LHJ, De Jong B and Oosterhuis JW (1995b) Cytogenetic evidence that carcinoma in situ is the precursor lesion for invasive testicular germ cell tumours. Cancer Genet Cytogenet 85,133–137.

Vierula M, Niemi M, Keiski A, Saaranen M, Saarikoski S and Suominen J (1996) High and unchanged sperm counts of Finnish men. Int J Androl 19,11–17.

Vogt PH (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet 5,933–944.

Vogt PH (2005) *AZF* deletions and Y chromosomal haplogroups: history and update based on sequence. Hum Reprod Update 4,319–336.

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

Von der Maase H, Giwercman A and Skakkebæk NE (1986) Radiation treatment of carcinoma in situ of testis. Lancet i,624–625.

von Eckardstein S, Syska A, Gromoll J, Kamischke A, Simoni M and Nieschlag E (2001) Inverse correlation between sperm concentration and number of androgen receptor CAG repeats in normal men. J Clin Endocrinol Metab 86,2585–2590.

von Eyben FE (2004) Chromosomes, genes, and development of testicular germ cell tumors. Cancer Genet Cytogenet 151,93–138.

Vos A, Oosterhuis JW, de Jong B, Buist J and Schraffordt Koops H (1990) Cytogenetics of carcinoma in situ of the testis. Cancer Genet Cytogenet 46,75–81

Wang PJ (2004) X chromosomes, retrogenes and their role in male reproduction. Trends Endocrinol Metab 15,79–83.

Wang PJ, McCarrey JR, Yang F and Page DC (2001) An abundance of X-linked genes expressed in spermatogonia. Nat Genet 27,422–426.

Weir HK, Marrett LD, Kreiger N, Darlington GA and Sugar L (2000) Pre-natal and peri-natal exposures and risk of testicular germ-cell cancer. Int J Cancer 87,438–443.

Williams K, McKinnell C, Saunders PT, Walker M, Fisher JS, Turner KJ, Atanassova N and Sharpe M (2001) Neonatal exposure to potent and environmental oestrogens and abnormalities of the male reproductive system in the rat: evidence for importance of the androgen-oestrogen balance and assessment of the relevance to man. Hum Reprod Update 7,236–247.

Xu EY, Moore FL and Reijo-Pera RA (2001) A gene family required for human germ cell development evolved from an ancient meiotic gene conserved in metazoans. Proc Natl Acad Sci USA 98,7414–7419.

Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, 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.

Yong EL, Loy CJ and Sim KS (2003) Androgen receptor gene and male infertility. Hum Reprod Update 9,1–7.

Yuasa T, Okamoto K, Kawakami T, Mishina M, Ogawa O and Okada Y (2001) Expression patterns of cancer testis antigens in testicular germ cell tumors and adjacent testicular tissue. J Urol 165,1790–1794.

Zamore PD and Haley B (2005) Ribo-gnome: the big world of small RNAs. Science 309,1519–1524.

Zeeman AM, Stoop H, Boter M, Gillis AJM, Castrillon DH, Oosterhuis JW and Looijenga LHJ (2002) VASA is a specific marker for both normal and malignant human germ cells. Lab Invest 83,159–166.

Zimmermann S, Steding G, Emmen JM, Brinkmann AO, Nayernia K, Holstein AF, Engel W and Adham IM (1999) Targeted disruption of the Insl3 gene causes bilateral cryptorchidism. Mol Endocrinol 13,681–691.

Zinn AR and Ross JL (2001) Molecular analysis of genes on Xp controlling Turner syndrome and premature ovarian failure (POF). Semin Reprod Med 19,141–146.

Submitted on February 3, 2005; resubmitted on December 21, 2005; accepted on January 10, 2006