

# Tumor Risk in Disorders of Sex Development

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## Key Words

Disorders of sex development · Germ cell tumors · Pathogenesis · Risk

## Abstract

Certain patients with disorders of sex development (DSD), who bear Y chromosome material in their karyotype, are at increased risk for the development of type II germ cell tumors (GCT), which arise from early fetal germ cells. DSD gonads frequently harbor immature germ cells which express early fetal germ cell markers. Some of them (e.g. OCT3/4 and NANOG) seem to be of pathogenetic relevance in GCT development providing cells with the ability of pluripotency, proliferation and apoptosis suppression. Also TSPY (testis-specific protein Y-encoded), the main candidate for the so-called gonadoblastoma locus on Y chromosome, is overexpressed in germ cells of DSD patients and possibly contributes to their survival and proliferation. Nowadays, the use of immunohistochemical methods is highly relevant in identifying DSD gonads at risk. The risk for GCT development varies. While the prevalence of GCT is 15% in patients with partial androgen insensitivity, it may reach more than 30% in patients with gonadal dysgenesis. Patients with complete androgen insensitivity and ovotesticular DSD develop ma-

lignancies in 0.8% and 2.6% of cases, respectively. However, these data may be biased for various reasons. To better estimate the risk in individual groups of DSD, further investigations on large patient series are needed.

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## Introduction

Disorders of sex development (DSD) are defined as congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical [Hughes et al., 2006]. The overall occurrence is estimated to be one in 4,500 live births [Hughes et al., 2006]. DSD represent a group of disorders with heterogeneous genetic background bringing a need of a uniform classification. Several attempts have been made in the past. The very last proposed classification is a result of consensus made by members of the European Society of Pediatric Endocrinology/Lawson Wilkins Pediatric Endocrinology Society (ESPE/LWPES) in Chicago in 2005 [Hughes et al., 2006].

Nowadays, every DSD patient should be provided with care of a skilled multidisciplinary team. Only under these circumstances, all specific issues of the different aspects of DSD are covered. Among these, the most pronounced

are sex assignment, psychological support, hormonal substitution, and reconstruction of external genitalia. In addition, in specific subgroups of DSD patients, namely those bearing Y chromosome material in their karyotype, prophylactic gonadectomy may be indicated due to an increased risk of gonadal tumor development [Nelson and Gearhart, 2004; Hughes et al., 2006; Mendonca et al., 2009]. Gonadectomy is an invasive and psychologically stressing procedure and may in some cases prevent the spontaneous course of puberty [Cools et al., 2006a]. The reason for increased tumor risk in DSD, the actual risk in the various subgroups and how to identify gonads at risk at an early stage are the topics of this review.

### Gonadal Tumors in DSD Patients

The overwhelming majority of gonadal neoplasias in DSD patients is represented by so-called type II germ cell tumors (GCT), namely classic seminoma in the testis and its counterpart dysgerminoma in the dysgenetic gonad as well as various types of nonseminoma/nondysgerminoma [Oosterhuis and Looijenga, 2005; Cools et al., 2006a]. Stromal cell tumors and epithelial tumors may also develop, although these are very rare [Verp and Simpson, 1987]. In this review, we will focus on the most frequent tumors, i.e. seminoma/nonseminoma, dysgerminoma/nondysgerminoma and their noninvasive precursors, carcinoma in situ (CIS) [Skakkebaek, 1972] and gonadoblastoma (GB), respectively [Cools et al., 2006b].

Based on their noticeable similarity in morphology and protein expression, it was suggested that the neoplastic cells (CIS and GB) are derived from fetal germ cells (primordial germ cells/gonocytes) arrested in an early stage of development [Dieckmann and Skakkebaek, 1999; Rajpert-De Meyts, 2006; Sonne et al., 2009].

While CIS is made up of neoplastic cells located within the context of seminiferous tubules, GB is composed of a mixture of different cell types including malignant germ cells and supporting (granulosa) cells arranged in well circumscribed nests (see fig. 1) [Kersemaekers et al., 2005]. Therefore, it was initially hypothesized that CIS and GB were 2 basically distinct entities. However, immunohistochemical profiling of both lesions indicates a tight similarity of their malignant cells. The difference in overall morphology may be only a result of a different gonadal microenvironment (see below for further discussion). Moreover, the theory of a common origin of CIS and GB is also supported by the resemblance of the subsequent invasive tumors, seminoma and dysgerminoma

respectively, in morphology, mRNA expression and miRNA profiling [Kersemaekers et al., 2005; Gillis et al., 2007; Hersmus et al., 2008a; Looijenga et al., 2010].

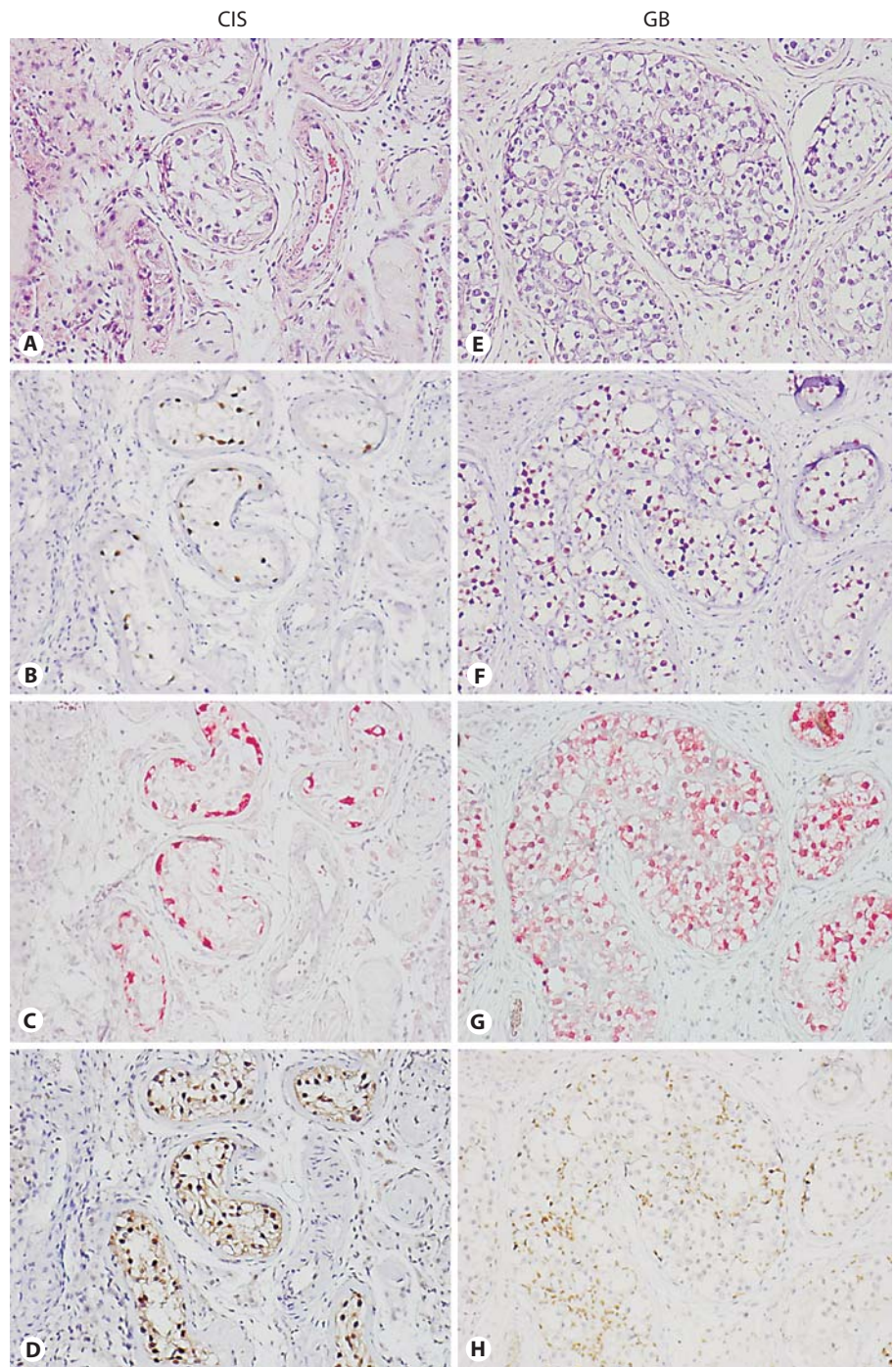
### Normal Gonadal and Germ Cell Development

To explain the relationship between DSD and GCT development more precisely, it is necessary to understand specific basic issues about the development of gonadal tissue and germ cells themselves. Our knowledge in this field is mainly based on research in mice but is, with some exceptions (see below), also applicable to humans.

Primordial germ cells (PGC) arise from embryonic stem cells (ESC) and can be detected in humans for the first time at 5–6 weeks of gestation in the yolk sac as they stain positive for PLAP (placental alkaline phosphatase) and OCT3/4 (octamer-binding transcription factor, also termed POU5F1), amongst others [Hersmus et al., 2008a; Looijenga et al., 2010]. OCT3/4 is a transcription factor which is physiologically expressed only in ESC and PGC to provide them with the ability of pluripotency, survival and proliferation [Cheng et al., 2006]. NANOG (Homeobox transcription factor NANOG), another pluripotency regulatory transcription factor, shows similar function and temporospatial expression as OCT3/4 [Hart et al., 2005; Hoei-Hansen et al., 2005]. Moreover, OCT3/4, as well as NANOG, prevents PGC from apoptosis [Kehler et al., 2004; Yamaguchi et al., 2009].

PGC migrate from the yolk sac along the hindgut towards the genital ridges. Among others, SCF (stem cell factor) and its receptor c-KIT are responsible for the proper migration. c-KIT is expressed in germ cells while SCF serves as chemo-attractant [Molyneaux and Wylie, 2004; Hersmus et al., 2008a]. As soon as the PGC arrive at the genital ridges, the structures are called indifferent gonads while PGC are termed gonocytes despite the unchanged morphology and expression profile [Looijenga et al., 2007].

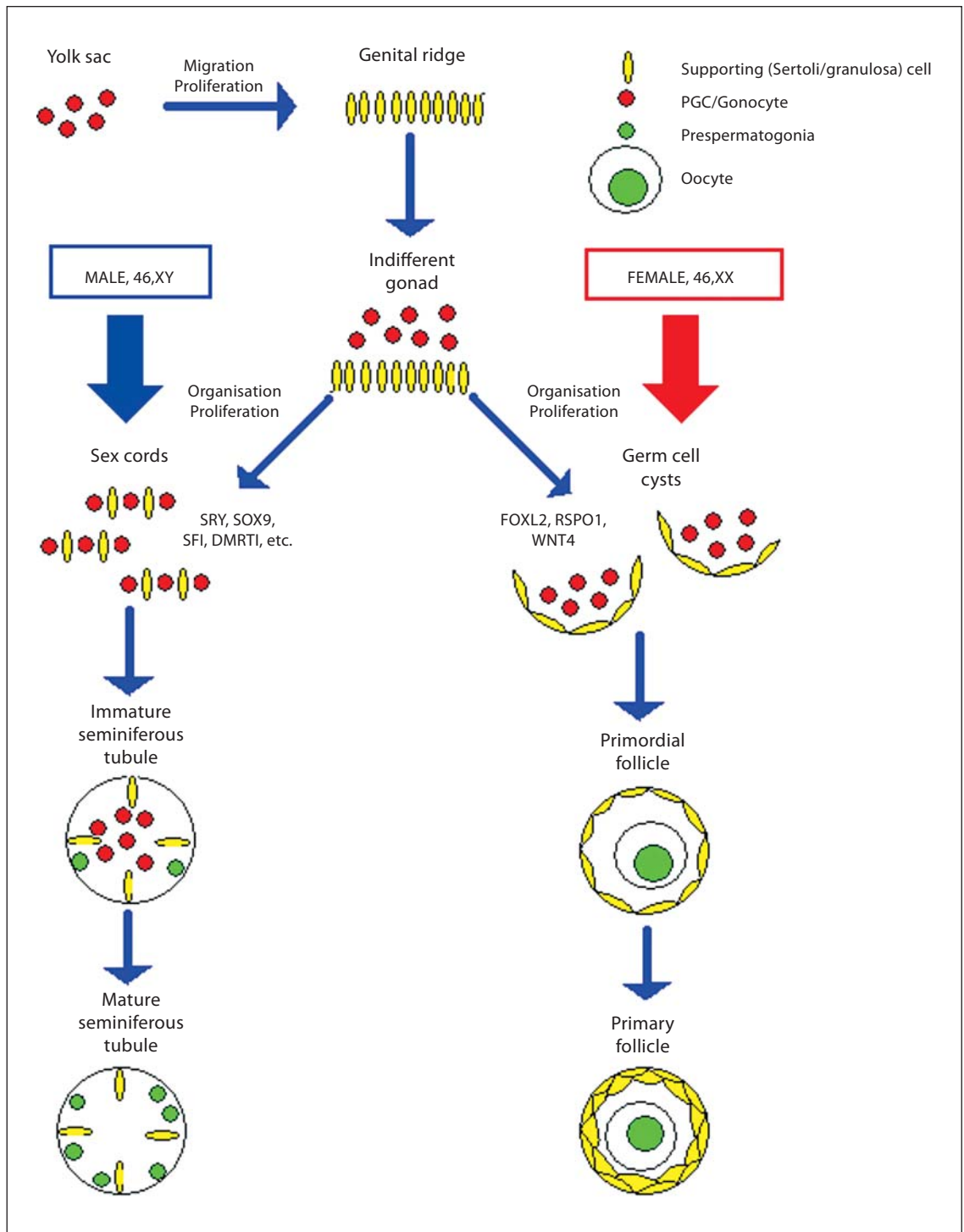
The indifferent gonad has a potential to develop either as a testis or as an ovary. Its fate depends on the genetic constitution, i.e. the combination of sex chromosomes (XY in males and XX in females) [Wilhelm et al., 2007]. In the presence of the Y chromosome, *SRY* (sex-determining region on Y, also *TDF*, i.e. testis-determining factor) expression occurs during human embryogenesis at week 7 of gestation in supportive (pre-Sertoli) cells and initiates the expression of a cascade of down-stream genes (among others *SOX9*) which orchestrate testicular differentiation [Fleming and Vilain, 2004; Wilhelm et al.,



**Fig. 1.** Carcinoma in situ (CIS) and gonadoblastoma (GB),  $\times 100$  magnification. **A** CIS: HE staining. **B** CIS: OCT3/4 positive malignant cells. **C** CIS: TSPY positive (malignant) germ cells. **D** CIS: SOX9 positive supporting (Sertoli) cells. **E** GB: HE staining GB. **F** GB OCT3/4 positive malignant cells. **G** GB: TSPY positive (malignant) germ cells. **H** GB: FOXL2 positive supporting (granulosa) cells.

2007]. During this process pre-Sertoli cells and gonocytes form cord-like structures (sex cords), and eventually seminiferous tubules [Wilhelm et al., 2007]. Initially, germ cells, still with all the characteristics of gonocytes, are located in the center of the tubules while Sertoli cells are situated in the periphery. Germ cells then start mi-

grating gradually towards the periphery. Once they reach the basal lamina, they mature to pre-spermatogonia, their morphology changes and expression of gonocyte markers (OCT3/4, NANOG, AP-2gamma, PLAP, c-KIT, etc.) ceases (see fig. 2) [Hoei-Hansen et al., 2004, 2005; Honecker et al., 2004; Pauls et al., 2006]. Testicular tissue



**Fig. 2.** Normal male and female gonadal differentiation. Primordial germ cells migrate from the yolk sac to the gonadal ridge which is then called indifferent gonad. Subsequent development can lead to either a male (sex cords, seminiferous tubules in the testicle) or a female (germ cell cysts, follicles in the ovary) differentiation pathway according to the chromosomal constitution and genes expressed.

of normal neonates hardly shows any OCT3/4 positive cells and none of these cells can be detected in 4-month-old infants [Looijenga et al., 2003b; Rajpert-De Meyts et al., 2004]. In mice, however, differences exist in expression pattern of the markers mentioned before in spermatogonia compared to humans [Lacham-Kaplan, 2004].

In females, absence of SRY expression allows the differentiation towards an ovary [Fleming and Vilain, 2004; Wilhelm et al., 2007]. Germ cells (oogonia) arrange in cyst-like structures with supportive (pre-granulosa) cells, then enter the first step of meiosis and form primitive follicles. At that time they lose OCT3/4 expression, and from then on they are called oocytes [Rajpert-De Meyts et al., 2004; Stoop et al., 2005; Wilhelm et al., 2007]. The process is no more believed to be a simple default pathway, since several indispensable genes (*FOXL2*, *WNT4*, *RSP01*) have been identified [Parma et al., 2006; Mandel et al., 2008; Uhlenhaut et al., 2009]. Recently, the importance of *FOXL2* in maintenance, instead of only induction, of ovarian phenotype of the gonad in mice was demonstrated, as the loss of *FOXL2* expression leads to up-regulation of *SOX9* and subsequently to a change of gonadal morphology, i.e. testis formation. *FOXL2* and *SOX9* are expressed in supporting cells (i.e. in granulosa and Sertoli cells, respectively) in a mutually exclusive manner [Hersmus et al., 2008b; Uhlenhaut et al., 2009].

### Pathogenesis of Germ Cell Tumors in DSD

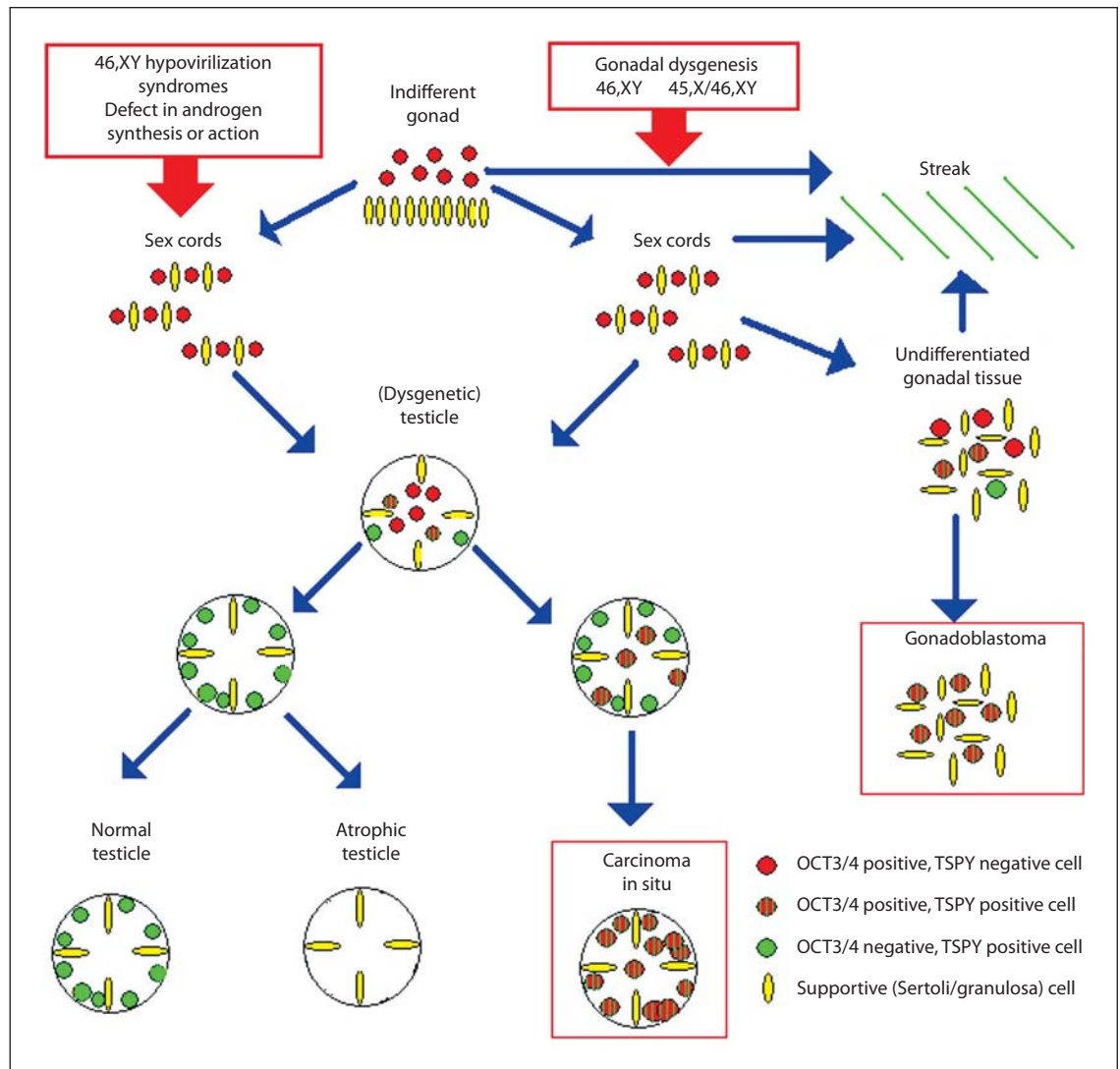
In gonads of DSD patients, immature germ cells which resemble PGC/gonocytes frequently persist as the insufficiently differentiated supporting cells (Sertoli/granulosa cells) are not able to provide a satisfactory milieu to induce maturation to either pre-spermatogonia or oocytes. Immature germ cells can be identified by the positivity for factors typically expressed in early fetal germ cells (OCT3/4, PLAP, etc.) [Cools et al., 2005]. The prolonged expression of OCT3/4 is believed to be one of the crucial factors in GCT development as it allows germ cells to survive and proliferate [Cools et al., 2009]. Thanks to its similar function and temporospatial expression pattern, NANOG supposedly has the same impact on tumorigenesis as OCT3/4.

Another piece of the GCT pathogenetic puzzle seems to be an abundant expression of TSPY (testis-specific protein Y-encoded) in germ cells in the DSD gonad. The physiological function of TSPY is not fully understood, but it is thought to be involved in the control of germ cell mitotic proliferation in normal testis [Lau et al., 2009].

Therefore, TSPY overexpression in germ cells may also contribute to their survival and proliferation in an unfavorable environment which would otherwise result in a depletion of the germ cell population. Interestingly, TSPY has no functional homologue in mice and no type II GCT has been reported in this species so far [Oosterhuis and Looijenga, 2005]. However, TSPY transgenic mice develop tumors of the pituitary gland and adrenals more frequently but not the gonadal tumors [Tascou et al., 2003]. In vitro TSPY potentiates cell proliferation by promoting cell cycle progression via cyclin B [Oram et al., 2006]. A special role of TSPY in GCT development in general, but in DSD patients specifically, is depicted by the fact that it is the most likely candidate for the GBY region (Gonadoblastoma locus on Y chromosome). Existence of such a gene was previously postulated by Page [1987] who based his hypothesis on genetic studies in DSD patients with GB or invasive tumor. As mentioned above, only patients with this part of the Y chromosome in their karyotype have a higher risk to develop a type II GCT.

The gonad in which the CIS/GB or GCT arise may be of various phenotypes. CIS and (non)seminoma develop in the context of testicular tissue both in patients with hypovirilization syndromes and with gonadal dysgenesis. GB seems to develop mainly in the context of undifferentiated gonadal tissue (UGT), which remarkably resembles the developmental stage of sex cords. Also streak tissue can harbor GB [Cools et al., 2006b]. Interestingly, supporting cells in GB display significant *FOXL2* (a marker for granulosa cells) and no or very low *SOX9* (Sertoli cell marker) expression, indicating that GB most likely arises in the gonads which failed to follow the male differentiation pathway (fig. 1) [Hersmus et al., 2008b]. The highest risk for GCT development is attributed to UGT and to the testis displaying dysplastic changes (see fig. 3) [Cools et al., 2009].

The forms of DSD with increased risk for GCT development could be considered as an extremely escalated case of testicular dysgenesis syndrome. Testicular dysgenesis syndrome was postulated as an umbrella entity for phenomena as testicular cancer, poor semen quality, undescended testicles, and hypospadias which frequently coincide in a single patient. The rapid increase of incidence of reproductive disorders indicates that environmental factors are the likely cause in most of the cases, although it is supposed that some genetic aberrations or polymorphisms might be involved [Skakkebaek et al., 2001; Wohlfahrt-Veje et al., 2009]. Indeed, genetic background in DSD patients seems to play a major role.



**Fig. 3.** Carcinoma in situ and gonadoblastoma development. In hypovirilization syndromes the gonad develops as a testicle, whereas in case of gonadal dysgenesis final morphology can vary from streak, undifferentiated gonadal tissue to a dysgenetic testicle according to the step of differentiation in which the development was disrupted. CIS arises from testicular tissue in which OCT3/4 positive germ cells persist and escape normal development while expressing TSPY at the same time. Similar persisting cells in UGT give rise to gonadoblastoma. Supporting cells in CIS and GB have characteristics of Sertoli and granulosa cells, respectively.

### The Risk for GCT Development Varies among Different Disorders

It is possible to estimate the risk for tumor development in various forms of DSD by analyzing previously reported patient series. However, with this approach we have to consider bias due to (i) inaccurate DSD classification, (ii) heterogeneous criteria for CIS/GB diagnosis, (iii) possibly preferential reporting of tumor cases, and (iv)

lower incidence in later series due to early prophylactic gonadectomy.

In the last few years, the most extensive meta-analysis was performed by Cools and colleagues [2006a]. They divided DSD patients into several risk groups for development of type II GCT. Patients with hypovirilization syndromes have a normal male karyotype and their gonads developed into normal testicles. These disorders are caused by a defect in either synthesis or action of andro-

gens. The most numerous are patients with androgen insensitivity syndrome. The overall prevalence of CIS and invasive type II GCT (seminoma and nonseminoma) in this group is estimated to 5.5%. There is, however, an important difference between patients with complete and partial androgen insensitivity syndrome in whom malignancies occur in 0.8% and 15%, respectively [Cools et al., 2006a]. Other hypovirilization syndromes are very rare. Malignancies in 17% of patients with 17 $\beta$ -hydroxysteroid dehydrogenase were mentioned in one small series [Cools et al., 2005], while only one case of seminoma in a patient with 5 $\alpha$ -reductase deficiency and no tumors in patients with Leydig cell hypoplasia have been reported so far [Sasaki et al., 2003; Cools et al., 2005]. However, this needs confirmation in larger series.

Patients with gonadal dysgenesis (with either a 46,XY or 45,X/46,XY karyotype) seem to be the most endangered subgroup, although the prevalence in different series is rather incoherent, being reported in 15–100% of all cases [Slowikowska-Hilczler et al., 2001; Cools et al., 2006a]. After the rational interpretation of available data, Cools et al. [2006a] rated the total occurrence at 12% and possibly at more than 30% if gonadectomy had not been performed. Particularly in patients with mosaic karyotype the prevalence ranges between 15 and 40%, while in those with 46,XY karyotype it achieves approximately 30%. In a series of patients with a defect of the *WT1* gene, malignancies were reported in 60% of patients with Frasier syndrome [Joki-Erkkilä et al., 2002] and in 40% of patients with Denys-Drash syndrome [Pelletier et al., 1991]. This knowledge is important because of the limitations on the use of chemotherapeutics for treatment of these patients based on their suboptimal kidney function.

In patients with ovotesticular DSD, previously referred to as true hermaphroditism, the occurrence of neoplasia is estimated at 2.6% (see table 1 for summary) [Cools et al., 2006a].

Patients with XX karyotype do not display a higher risk for development of GCT [Cools et al., 2009].

### How to Identify a Gonad at Risk for Development of Malignancy? The Role of OCT3/4, TSPY and SCF

From a clinical point of view, early diagnosis of gonads with increased risk of malignant transformation is mandatory, especially in patients with retained gonads. Beside close clinical follow-up, gonadal biopsy is of high relevance, although its representativeness for the complete gonad in DSD patients is a matter of debate [Cools et al.,

**Table 1.** Prevalence of type II GCT in various forms of DSD

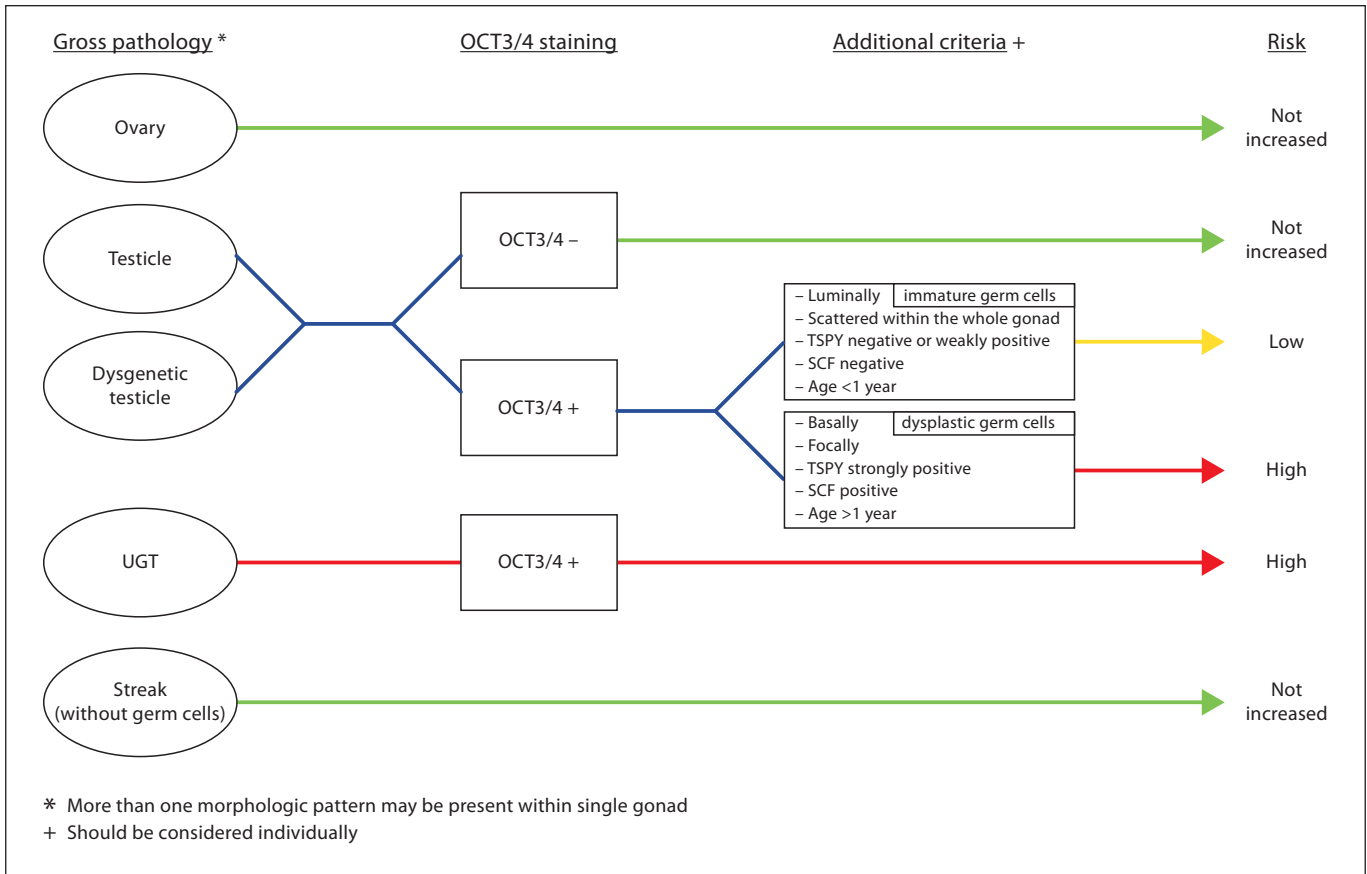
Risk	Type of DSD	Prevalence %
High	GD in general	12*
	46,XY GD	30
	Frasier syndrome	60
	Denys-Drash syndrome	40
	45,X/46,XY GD	15–40
Intermediate	PAIS	15
	17 $\beta$ -hydroxysteroid dehydrogenase deficiency	17
Low	CAIS	0.8
	Ovotesticular DSD	2.6
Unknown	5 $\alpha$ -reductase deficiency	?
	Leydig cell hypoplasia	?

GD = Gonadal dysgenesis; PAIS = partial androgen insensitivity syndrome; CAIS = complete androgen insensitivity syndrome.  
\* Might reach more than 30%, if gonadectomy has not been performed.

2009]. It is not expected that a GCT will arise in gonadal tissue in the absence of germ cells, as is the case in streak gonad and testis strictly formed by Sertoli cell only tubules. But even the gonad harboring germ cells may not be necessarily endangered if these do not display any sign of neoplastic changes (see fig. 4) [Looijenga et al., 2007]. The crucial diagnostic point then is to identify these cells at risk within the abnormally developed gonad.

In the last decades, several markers for detection of malignant germ cells in CIS/GB and GCT have been established (e.g. PLAP, c-KIT, OCT3/4, AP-2gamma, NANOG) [Looijenga et al., 2003b; Høe-Hansen et al., 2004, 2005, 2008; Honecker et al., 2004; Jones et al., 2004; de Jong et al., 2005]. Among these markers, OCT3/4 appears to be the most reliable for detection of CIS/GB in DSD gonads thanks to its consistent strong nuclear staining with weak or no background [Cools et al., 2005; Kersemaekers et al., 2005]. OCT3/4 is a transcription factor indispensable for maintenance of pluripotency in ESC and PGC. As discussed above, it seems to have an anti-apoptotic function in PGC [Kehler et al., 2004; Cheng et al., 2006]. It serves as a highly specific and sensitive marker for both CIS/GB and pluripotent types of GCT (i.e. seminoma/dysgerminoma and embryonal carcinoma) in which its expression is possibly of pathogenetic relevance [Looijenga et al., 2003b; Cools et al., 2009].

When assessing gonadal tissue in DSD patients, a more cautious approach should be applied due to possible

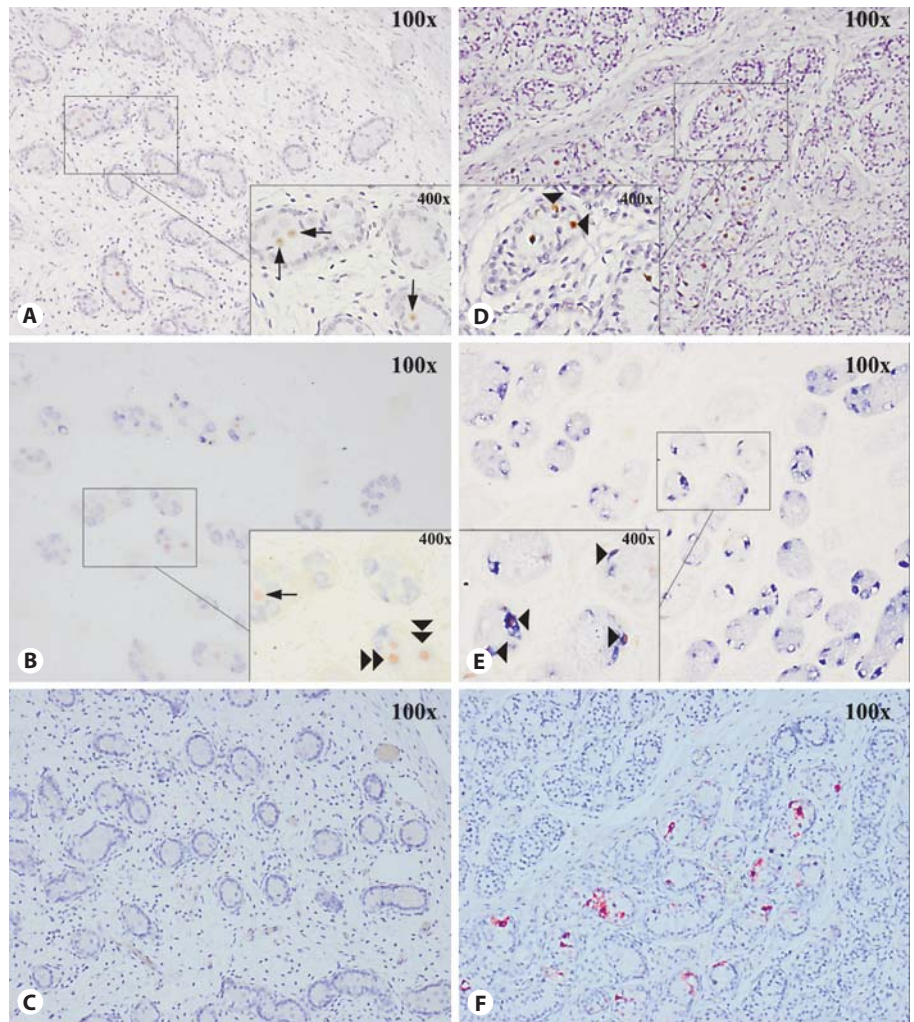


**Fig. 4.** Assessment of gonads at risk based on gross pathology and immunohistochemical staining. The risk for development of GCT is related to the pattern of gonadal differentiation. OCT3/4 positive germ cells in testis may be considered as immature or dysplastic according to the additional criteria. UGT, according to our experience, OCT3/4 positive cells are always being found within this morphologic pattern.

maturation delay of germ cells which frequently occurs in DSD patients and may lead to overdiagnosis and overtreatment [Cools et al., 2005]. Immature germ cells express similar markers as early fetal germ cells (PGC/gonocytes) and as CIS/GB cells which are believed to develop from them [Dieckmann and Skakkebaek, 1999; Cools et al., 2005; Rajpert-De Meyts, 2006]. Cools et al. [2005] proposed additional criteria which should help to distinguish between maturation delay and early neoplasia in patients with hypovirilization syndromes. The criteria are based on knowledge of fetal germ cell development. OCT3/4 positive cells which are located in the centre of the seminiferous tubules and are scattered throughout the whole gonad are believed to be delayed in their maturation, especially in patients younger than 1 year (see fig. 5A, D) [Cools et al., 2005].

As mentioned above, TSPY is the most probable candidate for GBY and is abundantly expressed in germ cells in DSD gonads and in CIS/GB cells [Schnieders et al., 1996; Cools et al., 2005; Kersemaekers et al., 2005; Lau et al., 2009]. During normal gonadal development TSPY expression is almost exclusively restricted to (pre-)spermatogonia, while OCT3/4 is expressed in gonocytes [Honecker et al., 2004; Pauls et al., 2006]. When migrating from the lumen towards the basal lamina of seminiferous tubules, germ cells gradually lose OCT3/4 expression whereas expression of TSPY becomes more intense during the maturation process. Double-staining for OCT3/4 and TSPY is informative for the detection of dysplastic cells, which escape normal development [Kersemaekers et al., 2005]. These cells are attached to basal lamina and continue strongly expressing both OCT3/4 and TSPY (as CIS/GB cells) [Cools et al., 2006a]. Double-positive cells





**Fig. 5.** Detection of dysplastic cells in a DSD gonad using immunohistochemistry. **A** OCT3/4 positivity in luminally located germ cells scattered within the gonad of a 16-month-old 46,XY female. **B** Same area with luminally located OCT3/4 (orange) positive cells showing no (arrow) or weak (double arrow heads) TSPY (blue) positivity. **C** Same area displaying SCF negativity. **D** Area of OCT3/4 positive basally located germ cells (arrow heads) in a 9-year-old 46,XY female. **E** Same area with OCT3/4 (orange) and TSPY (blue) double-positive germ cells attached to the basal lamina (arrow heads). **F** Same area displaying SCF positivity.

in the luminal site are likely to be only delayed in their maturation (fig. 5B, E).

Moreover, SCF seems to be a useful additional tool in distinguishing between immature germ cells and early malignant cells [Stoop et al., 2008]. SCF is a ligand of the proto-oncogene *c-KIT* which acts as a tyrosine kinase [Strohmeyer et al., 1995]. The SCF/*c-KIT* system plays an important role, not only in fetal germ cell migration as mentioned above but also in their proliferation and apoptosis as well as in regulation of adult spermatogonia proliferation and maintenance [Strohmeyer et al., 1995; Feng et al., 1999; Maduit et al., 1999; Hoei-Hansen, 2008; Stoop et al., 2008]. In the adult testis, SCF is synthesized by Sertoli cells in 2 splice variants – the membrane-bound form acts in establishing and maintaining germ cells, and the soluble form induces the testosterone production in Leydig cells [Maduit et al., 1999]. *c-KIT* mutations have been

identified in numerous GCT [Tian et al., 1999; Looijenga et al., 2003a; Biermann et al., 2007; Hoei-Hansen, 2008], and most recently, an association between specific polymorphisms within SCF and the risk for type II GCT has been reported [Kanetsky et al., 2009; Rapley et al., 2009], but still the detailed pathogenetic role of SCF in tumorigenesis remains to be elucidated. SCF is undetectable in germ cells of both normal fetal and postnatal testes using immunohistochemical methods. SCF staining in seminoma/dysgerminoma is heterogeneous and inconsistent in nonseminomas. However, SCF positivity in CIS/GB cells is convincing [Sandlow et al., 1996; Stoop et al., 2008]. Interestingly, in the DSD gonads, germ cells displaying OCT3/4 positivity but not fulfilling the criteria for CIS cells, i.e. cells with maturation delay, are SCF negative [Stoop et al., 2008]. This gives SCF a unique position among other CIS/GB markers (see fig. 5C, F).

## Looking towards the Future

Presently available tools allow us to assess gonadal tissue of DSD patients and identify gonads at risk for GCT development, i.e. gonads containing dysplastic cells or noninvasive neoplasia. This ability together with precise diagnosis of DSD cases based on molecular-genetic methods may facilitate a more accurate estimation of the tumor risk in various forms of DSD. With that knowledge we might be able to preserve gonads in selected patients.

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Of course, large series of patients are required for such an ambitious vision. As DSD is relatively rare, multi-centric studies and international cooperation are indispensable.

## Acknowledgements

This work was supported in part by ESPE Research Fellowship, sponsored by Novo Nordisk A/S (J.P.) as well as the Translational Research Grant Erasmus MC 2006 (R.H.) and The Flanders Research Foundation-FWO (M.C.).

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