

Neoplastic Potential of Germ Cells in Relation to Disturbances of Gonadal Organogenesis and Changes in Karyotype

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ABSTRACT: The study consisted of 46 intersexual patients who underwent gonadectomy at the age of 3 months to 19 years because of gonadal dysgenesis (GD; 40 cases) or true hermaphroditism (bisexual gonads; 6 cases). In patients with GD, the incidence of the 46,XY karyotype was 67.5%, whereas the remaining patients exhibited numerical and structural aberrations of sex chromosomes (NSASs), and all patients with bisexual gonads revealed NSAS. Seminoma was diagnosed in 1 patient with the 46,XY karyotype and pure GD (streak gonads). Intratubular carcinoma in situ (CIS) appeared as an exclusive lesion in 61.5% of 13 patients with mixed GD, in 54% of 11 patients with partial GD (bilateral testes), in 16.7% of 6 patients with bisexual gonads, and in none of 13 patients with pure GD. CIS also appeared in tubules in the vicinity of sex cord-derived tumors (gonadoblastoma nests and unclassified mixed germ cell-sex cord-stromal tumor; MGCSCST) and within the tumors. In 3 patients, gonadoblastoma replaced the whole bilateral gonads and is referred to as gonadoblastoma-only GD. The incidence of neoplastic lesions (mostly bilateral) was 90.9% in patients with partial GD, 76.9% (mostly unilateral) in patients with mixed GD, 23.1% (unilateral) in patients with pure GD, and 16.7% (unilateral) in patients

with bisexual gonads. Disregarding types of disturbances of gonadal organogenesis, the incidence of lesions was 71.4% in 28 patients with the 46,XY karyotype and 35.3% in 17 patients with NSAS. We conclude, first, that NSAS is not a prerequisite for the appearance of GD and GD is more frequently associated with the 46,XY karyotype. Second, the spectrum of germ cell neoplastic lesions in GD is wider than reported. Besides germ cell carcinoma, CIS, and gonadoblastoma nests, the spectrum also includes a tumor of gonadoblastoma-only in cases of GD and MGCSCST. Third, the incidence of neoplastic lesions is related more to the severity of the disturbances of gonadal organogenesis than it is to aberrations in sex chromosomes. Fourth, less disturbed testicular organogenesis predisposes these patients more toward germ cell neoplastic lesions, which suggests that the testicular environment of a dysgenetic gonad plays an important role in germ cell neoplasia initiation, maintenance, or both.

Key words: Testicular carcinoma in situ, gonadoblastoma, mixed germ-cell sex cord stromal tumor, gonadal dysgenesis, sex chromosomes.

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The postnatal presence of abnormal germ cells in seminiferous tubules, similar in appearance to gonocytes, is considered pathologic and is termed testicular carcinoma in situ (CIS) (Jørgensen et al, 1993). It has been generally accepted that CIS arises from gonocytes during fetal development of the testis (Teter, 1960; Skakkebaek, 1972; Skakkebaek et al, 1987). Assuming that CIS is a premalignant form of the primordial germ cells, it is probable that aneuploidy in fetal germ cells acts as the initial step in utero before the primordial germ cells migrate from the yolk sac to the left and right gonadal blastema. This is one possible explanation for the incidence of bilateral germ cell carcinoma (Dieckman and Loy, 1993).

Abnormalities in the organogenesis of gonadal blastema may participate in the initiation and progression of

germ cell tumors. In particular, patients with gonadal dysgenesis (GD) have the greatest risk for developing germ cell tumors (Verp and Simpson, 1987). Among 70 patients from different high-risk groups of germ cell carcinoma, the incidence of CIS cells located in seminiferous tubules (intratubular CIS) was highest in children with GD (Słowikowska-Hilczer et al, 2001b). In these children, 42%–97% of CIS cells revealed aneuploid DNA content and high proliferative potential (Słowikowska-Hilczer, 2001). This implies that fetal germ cells, which are present in testes with disturbed organogenesis, are susceptible to replication errors during mitosis, resulting in a gain or loss of chromosomes, and that these cells should be considered neoplastic. In addition to CIS, germ cell neoplastic lesions include the sex cord-derived tumors, gonadoblastoma and unclassified, mixed germ cell-sex cord stromal tumor (MGCSCST) (Talerman, 1980). Unlike gonadoblastoma, which occurs in GD, MGCSCST occurs mostly in the ovaries of phenotypic women with the 46,XX karyotype. However, a few testicular unclassified MGCSCSTs in otherwise healthy men have also been re-

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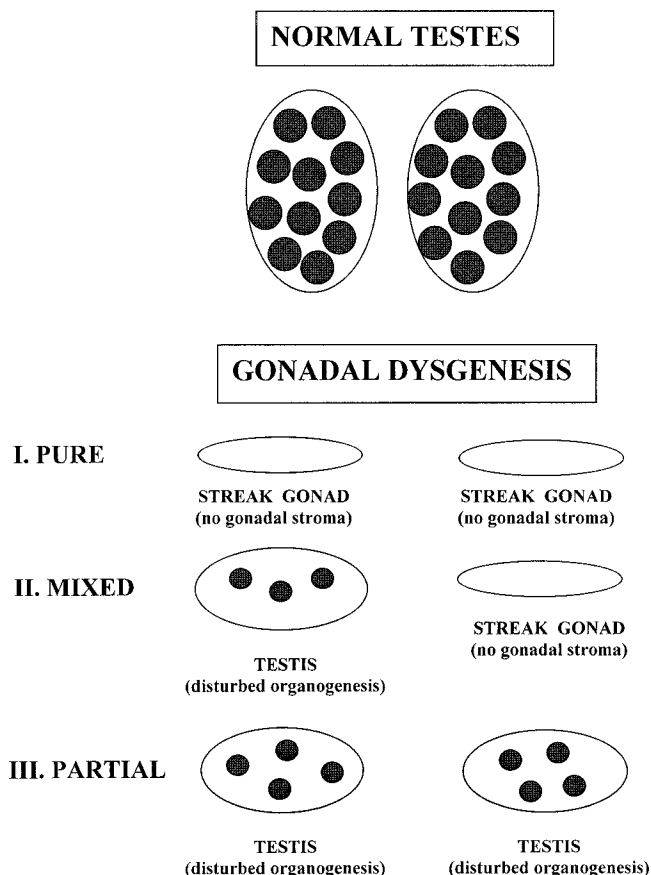


Figure 1. Schematic representation of the classification of GD. (I) Pure GD, in which only streaks of connective tissue are present bilaterally. (II) Mixed GD, in which seminiferous tubules (black circles) are rarely distributed on one side (dysgenetic testis) and a streak of connective tissue represents a gonad on the other side. (III) Partial GD, in which seminiferous tubules are rarely distributed in both gonads (dysgenetic testes on both sides). In all types, gonads are located abdominally in the ovarian position or, less frequently, in the upper segment of the inguinal canal.

ported (Talerman, 1980; Bolen, 1981; Matoska and Talerman, 1989; Rames et al, 1995; Ulbright et al, 2000).

GD covers a spectrum of disturbances in the organogenesis of the gonads, presumably due to different numerical and structural aberrations of sex chromosomes (NSASs). Consequently, a role for NSAS in gonadal tumorigenesis has been postulated (Müller, 1985; Müller et al, 1999; Sarafoglou and Ostrer, 2000). In this study we attempt to investigate germ cell neoplastic lesions in gonads of intersexual patients with GD and true hermaphroditism, and aimed to ascertain whether lesion occurrence can be related to the severity of disturbances in gonadal organogenesis or to specific karyotype aberrations.

Materials and Methods

Patients

Forty-six intersexual patients aged 3 months to 19 years were investigated. Ambiguous external genitalia were present in 29

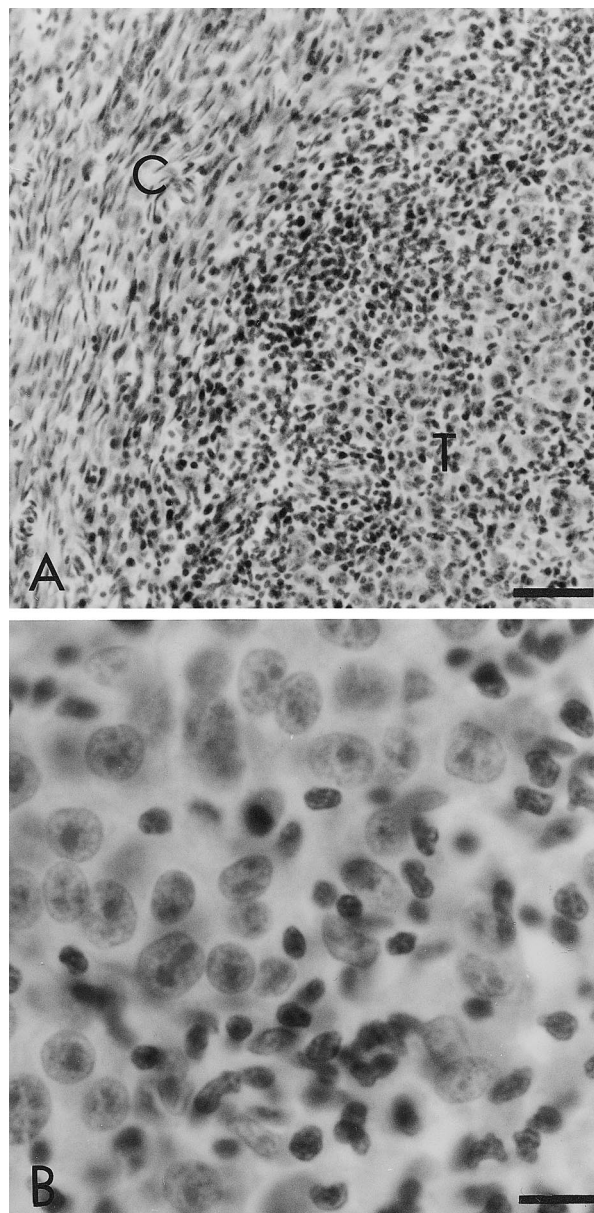


Figure 2. Seminoma in the gonad of a 46,XY 17-year-old patient with pure gonadal dysgenesis. (A) A fragment of the tumor (T) surrounded by connective tissue (c). (B) The tumor is composed of closely packed neoplastic germ cells with large nuclei and coarse clumps of chromatin resembling fetal germ cells, but with more pronounced nuclear polymorphism. Lymphocytic infiltration is visible. Scale bars = 80 μm (A), 20 μm (B).

patients, whereas female genitalia were present in 17. All had female internal sex organs (uterus and oviducts) as revealed by ultrasonography and genitography. Cytogenetic diagnosis was made using a standardized analysis of G banded chromosomes from peripheral blood lymphocytes. Fifty to 80 mitoses were examined to determine the proportion of the cell line mosaic.

Bilateral gonadectomy was performed for all patients in order to prevent germ cell carcinoma. During surgery, gonads were found either bilaterally in the abdomen in the position typical for an ovary, or in the upper segment of the inguinal canal.

Table 1. Incidence of karyotypes and neoplastic lesions in the gonads of patients with disturbances of gonadal organogenesis

Disturbance	Karyotype	n	(%)	Number of neoplastic lesions
Pure GD (streak gonads)	46,XY	9	(69.2)	2 (seminoma, GDA nests)
	45,X/46,XY	3	(33.1)	1 (GDA nests)
	45,X/46,X,+mar(Y)	1	(7.7)	0
Mixed GD (unilateral testis)	46,XY	10	(76.9)	9 (CIS, GDA nests, MGCSCST)
	45,X/46,XY	1	(7.7)	0
	45,X/46,X,+mar(Y)	1	(7.7)	0
	45,X/47,XXY	1	(7.7)	1 (CIS)
Partial GD (bilateral testis)	46,XY	6	(54.5)	6 (CIS, GDA nests, MGCSCST)
	46,XX	1	(9.1)	1 (CIS)
	45,X/46,XY	2	(18.2)	1 (CIS, MGCSCST)
	45,X/46,XYq-	1	(9.1)	1 (CIS)
	46,XY, t(7;9)	1	(9.1)	1 (CIS, GDA nests)
Gonadoblastoma-only GD	46,XY	2	(66.7)	2 (GDA instead gonads)
	45,X/46,X,del(Y)(q12)	1	(33.3)	1 (GDA instead gonads)
Ovotestis/ovary	45,X/46,Xt(Yp;Xp)	1	(16.7)	1 (CIS)
Ovotestis/testis	45,X/46,XX/47,XXY	1	(16.7)	0
Ovotestis/testis	46,XX*	1*	(16.7)	0
Testis/ovary	46,XX*	1*	(16.7)	0
Testis/ovary	46,XX/46,XY	1	(16.7)	0
Testis/ovary	46,XX/47,XXY	1	(16.7)	0

* Indicates positive for the SRY gene; the Yp fragment was translocated onto a late-replicating, inactive X chromosome (Kusz et al., 1999).

Histopathology

Tissues were fixed in Bouins solution and embedded in paraffin. Each gonad was sectioned serially in its entirety into 5- μ m-thick slices. Several slides were stained with hematoxylin and eosin for histologic examination.

Gross histologic diagnosis (Fig. 1) was categorized on the basis of established nomenclature: 1) pure GD, bilateral streak gonads (no gonadal stroma); 2) mixed GD, a streak of connective tissue on one side and testis-like stroma on the other (unilateral gonad); 3) partial GD, bilateral testes with incomplete organogenesis; or 4) bisexual gonads, either bilateral ovotestis or testis and ovary in the same individual (Nezelof, 1991; Berkovitz and Seherunvong, 1998).

Intratubular CIS, gonadoblastoma, MGCSCST, and seminoma were recognized on the basis of their morphological features. For CIS cells these criteria include a nucleus irregular in shape and >7.5 μ m in diameter; irregular, coarse clumps of chromatin; and light and abundant cytoplasm (Müller, 1987).

Gonadoblastoma consists of collections of cellular nests surrounded by connective tissue. The nests are a mixture of CIS cells and somatic cells that resemble immature Sertoli or granulosa cells (Scully, 1970).

MGCSCST consists of germ cells admixed with sex cord elements, and therefore it has a similar cellular composition to that of gonadoblastoma. The tumor may exhibit 3 different histological patterns: 1) narrow cords that expand in places to form large round or oval cellular aggregates surrounded by connective tissue; 2) collections of solid tubules devoid of a lumen and surrounded by fine fibrovascular septa; or 3) large, solid aggregates devoid of any specific arrangement (Krag Jacobsen and Talerman, 1989).

Seminoma (gonocytoma or dysgerminoma) is composed of fetal germ cells. The morphology is identical to that of CIS cells.

The cells form aggregates, islands, cords, and strands all surrounded by varying amounts of fibrous connective tissue. The connective tissue is invariably infiltrated with lymphocytes and usually also with plasma cells, histiocytes, granulocytes, and eosinophils (Krag Jacobsen and Talerman, 1989).

Immunohistochemistry

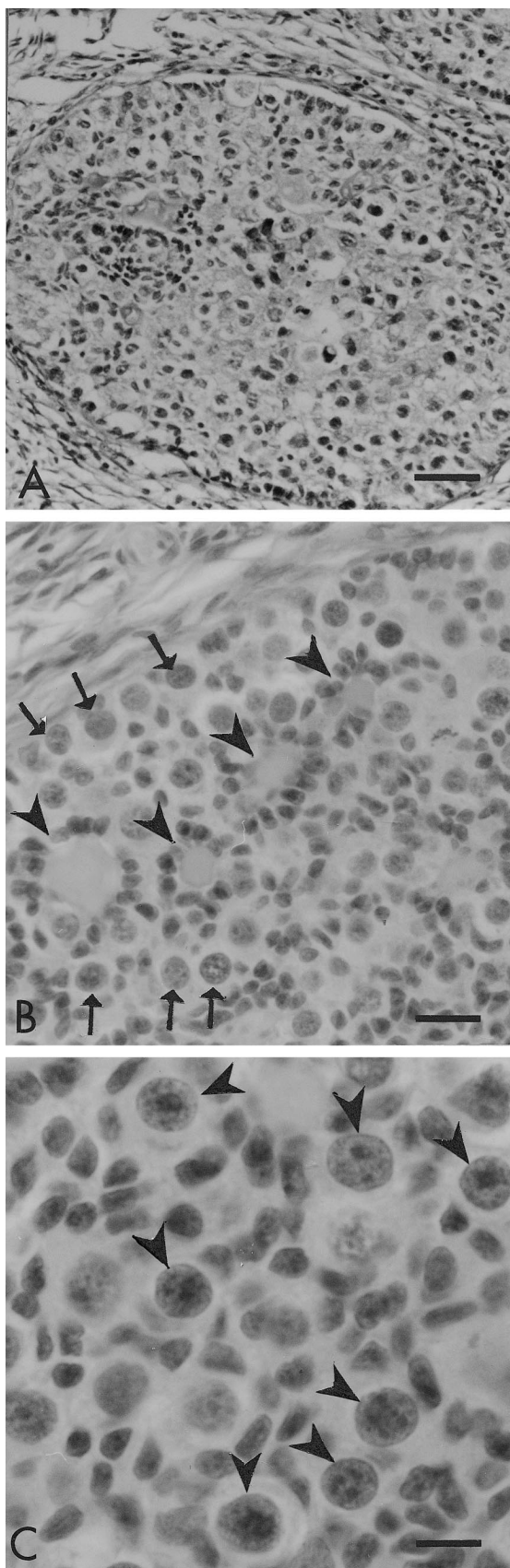
The presence of placental-like alkaline phosphatase antigen (PLAP) can be demonstrated by immunohistochemistry in tumor cells of seminoma as in CIS cells and fetal gonocytes (Skakkebaek et al, 1987). PLAP antigen is absent in postnatal spermatogenic cells, beginning from normal spermatogonia onward.

To detect the expression of PLAP, we performed an immunohistochemical examination as described previously (Słowikowska-Hilczer, 2001). In short, 5 sections from each gonad were treated with polyclonal antibody against PLAP (DAKO, Copenhagen, Denmark) diluted 1:100 in 0.05 M Tris-buffered saline pH 7.4. The method involved the peroxidase-antiperoxidase technique. 3,3'-Diaminobenzidine was used as a chromogen. All sections were counterstained with Mayers hematoxylin. Paraffin-embedded sections of testicular seminoma served as a positive control. For the negative control, the primary antibody was replaced with 0.05 M Tris-buffered saline.

Results

Patients, and Gross Histology of Gonads and Annexed Organs

Microscopic investigations revealed GD in 40 patients and bisexual gonads in 6 patients. According to clinical data, dysgenetic male pseudohermaphroditism was diag-



nosed in patients with GD, and true hermaphroditism was diagnosed in those with bisexual gonads.

Table 1 shows that among 40 patients with GD, an equalized proportion of pure GD (13 patients), mixed GD (13 patients), and partial GD (11 patients) was found. In 24 patients (60%), gonads consisted of testis stroma (mixed and partial GD). In 3 patients, bilateral gonadoblastoma entirely replaced the gonads. The sex of gonadal stroma was undefined in these patients, so the gonads were assigned as gonadoblastoma-only GD. In those with true hermaphroditism, 3 patients had a testis on one side and an ovary on the other, 2 displayed ovotestis with contralateral testis, and 1 patient displayed ovotestis on one side with a contralateral ovary.

In organs annexed to the gonads, fallopian tube structures were confirmed histologically in all cases. Wolffian duct structures were found in 11 of 13 (84.6%), 9 of 13 (69.2%), and 10 of 11 patients (90.9%) with pure, mixed, and partial GD, respectively. In gonadoblastoma-only GD, organs were found in 1 of 3 cases (33.3%), and in patients with bisexual gonads, organs were found in 2 of 6 cases (33.3%).

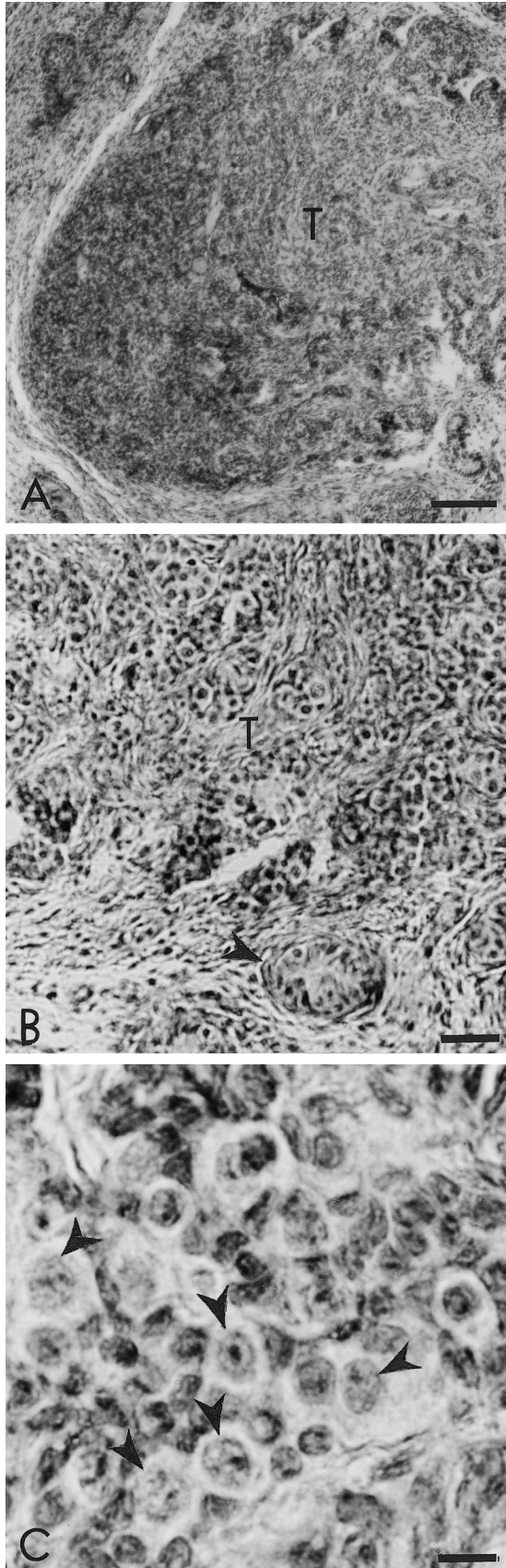
Gonadectomy was performed at age 3 to 19 years (mean 12.8 ± 1.4 , median 5 years) in those with pure GD, at 3 months to 15 years (mean 3.7 ± 1.0 , median 3 years) in those with mixed GD, and at 3 months to 8 years in those with partial GD (mean 2.3 ± 0.6 , median 2 years). The ages of 3 patients with gonadoblastoma-only GD was 7, 12, and 16 years; patients with true hermaphroditism underwent gonadectomy at age 1.5 to 14 years (mean 6.9 ± 3.3 , median 8 years). Thus, gonadectomy was performed on patients with pure GD, gonadoblastoma-only GD, and true hermaphroditism at a later age than it was in the remaining patients. This was probably due to an unequivocal female phenotype and female sexual identification. It is for this reason that referral to a pediatric endocrinologist probably occurred at a later age.

Disturbances in Gonadal Organogenesis Versus Karyotype

Table 1 indicates that among 40 individuals with GD, 27 had the 46,XY karyotype (67.5%). In patients with pure GD, the incidence of 46,XY was 69.2%, in those with mixed GD it was 76.9%, and in those with partial GD the

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Figure 3. Gonadoblastoma nest in a 46,XY 7-year-old patient with mixed gonadal dysgenesis. (A) Note the clear delineated boundary membrane of the tumor nest. (B) Bodies of hyalinization (arrowheads) are surrounded by somatic immature Sertoli/granulosa cells localized among numerous CIS cells (arrows). (C) Nuclear polymorphism of the germ cell compartment is similar to that in seminoma (see Fig. 2B), however, CIS cells (arrowheads) are separated by somatic cells. Scale bars = 80 μ m (A), 40 μ m (B), 20 μ m (C).



incidence was 54.5%. The 45,X/46,XY mosaicism was the most frequent type of NSAS. In the group of individuals with bisexual gonads (true hermaphroditism) 2 patients had the 46,XX karyotype. DNA analysis with fluorescence in situ hybridization revealed that both patients were positive for the *SRY* gene. The Yp fragment was found to be translocated onto a late-replicating, inactive X chromosome in both patients (Kusz et al, 1999). The remaining 4 individuals had NSAS. Therefore, all individuals with bisexual gonads revealed abnormalities in sex chromosomes with the presence of Y chromosome, or Y chromosome “material.”

Disturbances in Gonadal Organogenesis Versus Germ Cell Neoplastic Lesions

Early stage seminoma (invasive germ cell carcinoma) was present in 1 patient with the 46,XY karyotype, and the individual underwent gonadectomy at 17 years of age. The tumor was 1.5 mm in diameter, it had appeared unilaterally in one of the abdominal gonads (Figure 2), and was diagnosed as pure GD. Focally developed sex cord-derived tumors as gonadoblastoma nests (Figure 3) and MGCSCST (Figure 4), together with a tumor of gonadoblastoma-only GD, were present in 11 of 40 individuals with GD (27.5%) and in none with bisexual gonads.

Table 2 associates different types of the disturbance in gonadal organogenesis with the incidence of specific germ cell neoplastic lesions. The most frequent lesion was intratubular CIS (Figure 5), appearing as an exclusive lesion in 61.5% of 13 patients with mixed GD, 54.5% of 11 patients with partial GD, in 16.7% of 6 patients with bisexual gonads, and in none of 13 patients with pure GD. Intratubular CIS occurred in only 1 individual with bisexual gonads in the testicular compartment of ovotestis (Figure 6). Furthermore, CIS appeared in tubules that were located in the vicinity of focally developed sex cord-derived tumors (gonadoblastoma nests and MGCSCST) and within the tumors, including gonadoblastoma-only GD. Excluding tumor gonadoblastoma-only GD, the incidence of the entire branch of germ cell neoplastic lesions was 90.9% in patients with partial GD, 76.9% in patients with mixed GD, 23.1% in patients with pure GD, and 16.7% in those with bisexual gonads (Table 2).

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Figure 4. Unclassified mixed germ cell-sex cord stromal tumor (MGCSST) in a 46,XY 3-year-old patient with partial gonadal dysgenesis. (A) A conical tumor (T) with dimensions about 1.2×1.8 mm. (B) Note the presence of a seminal tubule cross-section (arrowhead) in the vicinity of the tumor. (C) Higher magnification of MGCSST revealed a mixture of CIS cells (arrowheads) and immature Sertoli/granulosa cells similar in appearance to the cellular arrangement of gonadoblastoma, but not composing distinct nests. Scale bars = 200 μ m (A), 40 μ m (B), 20 μ m (C).

Table 2. Incidence of germ cell neoplastic lesions

Disturbance	Germ cell tumor n (%)	Intratubular CIS n (%)	Sex cord tumor n (%)	Intratubular CIS+ sex cord tumor n (%)	Total n (%)
Pure GD (n = 13)	1 (7.7)	0	2 (15.4)	0	3 (23.1)
Mixed GD (n = 13)	0	8 (61.5)	0	2 (15.4)	10 (76.9)
Partial GD (n = 11)	0	6 (54.5)	0	4 (36.4)	10 (90.9)
Gonadoblastoma-only GD (n = 3)	0	0	3 (100.0)	0	3 (100.0)
Bisexual gonads (n = 6)	0	1 (16.7)	0	0	1 (16.7)

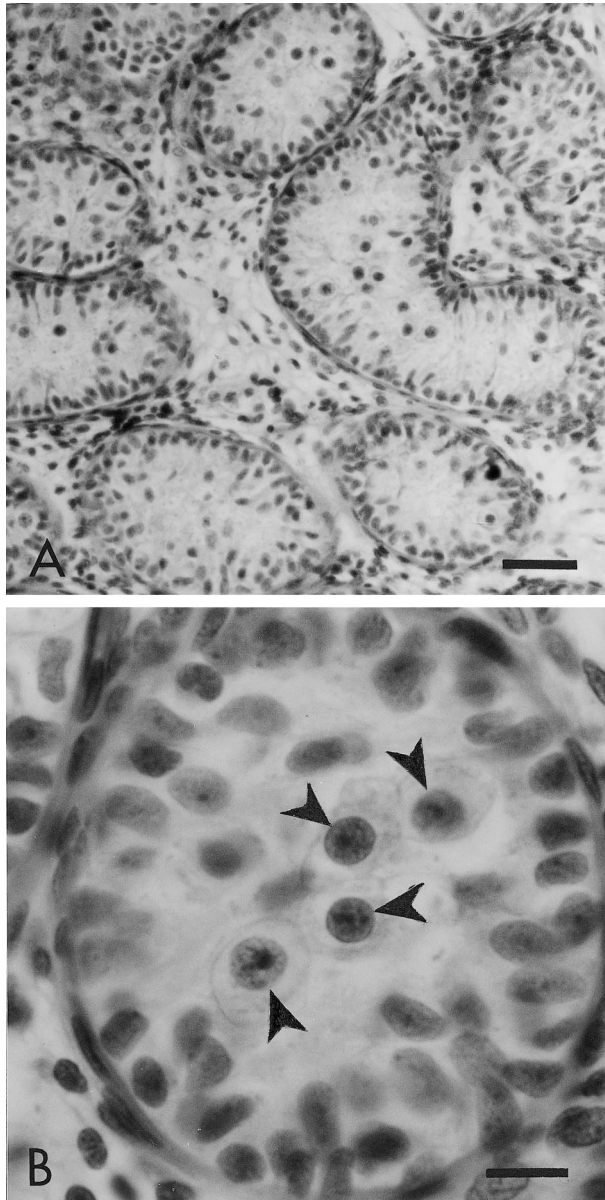


Figure 5. Partial gonadal dysgenesis in a 3-year-old patient with 46,XY karyotype. (A) Note the apparently normal structure of the testis. (B) Higher magnification reveals, however, the lack of spermatogonia. Instead, numerous CIS cells are present in the lumen of the tubule cross-section (arrowheads). Elongated nuclei of Sertoli cells indicate their immature character. They are located close to the basement membrane. Scale bars = 80 μ m (A), 20 μ m (B).

Germ Cell Neoplastic Lesions Versus Karyotype

The patient with seminoma had the 46,XY karyotype. Table 3 demonstrates that gonadoblastoma-only GD was found in 2 patients with 46,XY and in 1 with NSAS. Focally developed gonadoblastoma nests were found in 3 patients with 46,XY, 1 patient with 45,X/46,XY, and in 1 patient with 46,XY and autosomal translocation 7;9. Focal MGCSCST was diagnosed in 3 individuals with 46,XY and in 1 with 45,X/46,XY. Altogether, the 46,XY karyotype was present in 7 of 11 patients (63.6%) with sex cord-derived tumors.

Intratubular CIS, appearing as an exclusive neoplastic lesion, was present in 1 individual with 46,XX and partial GD (Table 1), but it was more frequent in those with 46,XY (12 of 28 cases, 42.9%) (Table 4). Table 4 shows the incidence of all germ cell neoplastic lesions associated with karyotype. One individual with the 46,XX karyotype and partial GD, and lacking Y chromosome DNA analysis was not included. The 46,XY karyotype and neoplastic lesions were present in 20 of 28 patients (71.4%), whereas NSAS was present in 6 of 17 patients (35.3%).

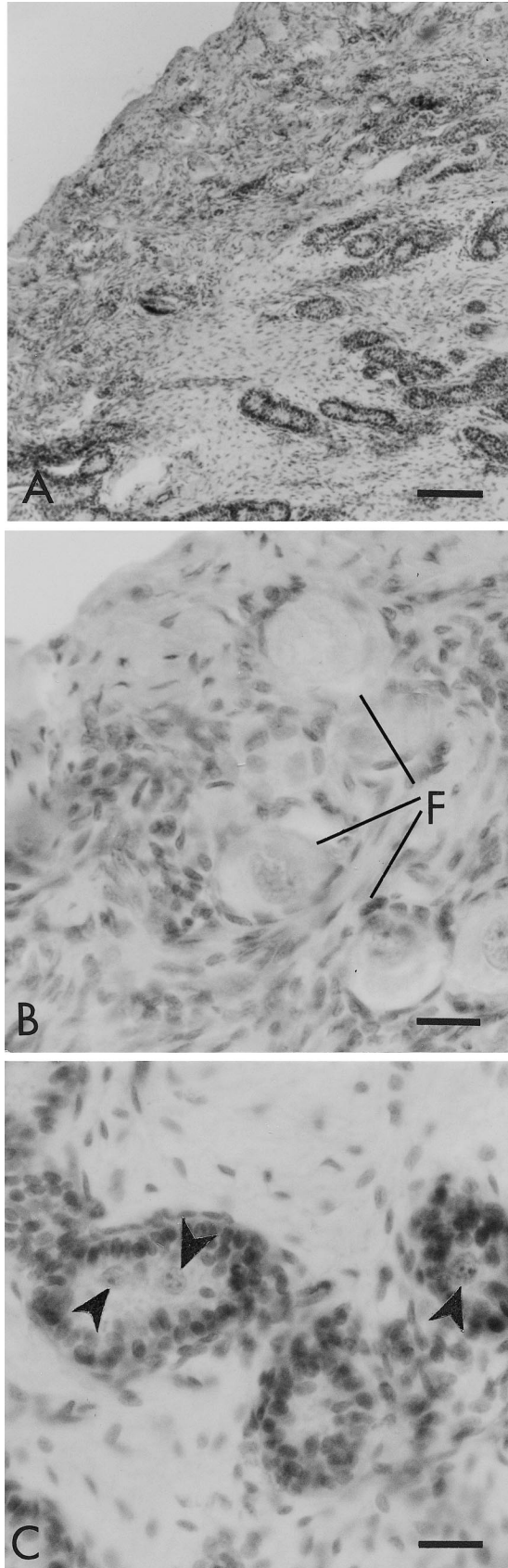
Laterality of Germ Cell Neoplastic Lesions Versus Type of Gonadal Dysgenesis or Karyotype

Table 3 indicates that whereas gonadoblastoma-only GD occurred bilaterally in 3 individuals, focal gonadoblastoma nests appeared unilaterally. MGCSCST was unilateral in 3 individuals and bilateral in 1 individual.

The elaboration presented in Table 5 does not include patients with bisexual gonads and gonadoblastoma-only GD. One individual with 46,XX and partial GD was also not included. The table shows that the bilateral appearance of germ cell neoplastic lesions was more frequent (70% of cases) in gonads with less impaired testicular organogenesis (partial GD) than in more severe types (ie, pure and mixed GD). In patients with NSAS, neoplastic lesions occurred unilaterally in 80% and bilaterally in 20% of cases, however, the number of patients with NSAS and was relatively low.

Discussion

To our knowledge, this is one of the largest clinical reports to associate the incidence of germ cell neoplastic



lesions with gross pathology of gonads and karyotypes. We showed that GD occurs most frequently with the 46,XY karyotype rather than with NSAS, as had been believed. According to reports by Müller (1985), Müller et al (1999), and Sarafoglou and Ostrer (2000), GD develops as a result of the mosaicism of sex chromosomes, mostly 45,X/46,XY. The clinical material in those reports was, however, scant. We also showed that less impaired organogenesis of the testis (partial and mixed GD) predisposes more toward the occurrence of germ cell neoplastic lesions than severe GD (streak gonads), and that less impaired organogenesis of the testis predisposes more toward bilateral occurrence of lesions. These findings suggest that testicular environment may play a pivotal role in the initiation of germ cell neoplastic lesions in GD. Although invasive germ cell carcinoma (seminoma) was diagnosed in a 17-year-old 46,XY patient with streak gonads, it is likely that the gonad was a testis before tumor formation began.

We described 4 cases of MGCSCST, which occurred predominantly with the 46,XY karyotype (3 cases). Until now, MGCSCST has never been described in GD, but 10 cases of testicular MGCSCST in otherwise normal adult men have been reported (Ulbright et al, 2000). Similarly, to our knowledge, this study is the first to describe a tumor of gonadoblastoma-only GD that developed bilaterally instead gonad development. Again, more patients with this tumor had the 46,XY karyotype than had NSAS. We found also that CIS cells were localized not only within MGCSCST, but also inside seminiferous tubules in the vicinity of the tumor. This suggests a more generalized pathology of the testes when MGCSCST appears in children with GD.

CIS was the most abundant neoplastic lesion, appearing in 3 forms: as exclusive intratubular lesions, as a lesion associated with sex cord-derived tumors in the same gonad, and as a lesion located within sex cord-derived tumors. It appears, therefore, that besides intratubular CIS, CIS cells may be a constituent of a malignant potential of all sex cord-derived tumors, as was previously postulated for gonadoblastoma by Jörgensen et al (1997).

Intratubular CIS was observed more frequently with the presence of 46,XY (40.7%) than with NSAS (17.6%). Our earlier study showed that intratubular CIS cells, appearing in patients with GD and 46,XY, were neoplastic germ cells. In particular, they were aneuploid

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Figure 6. Ovotestis (true hermaphroditism) in a 2-year-old child with 45,X/46,Xt(Yp;Xp). (A) Two distinct compartments of the gonad are visible. The cortical one is the ovarian stroma and the medullary compartment is the dysgenetic testis. (B) The ovarian compartment shows primary follicles (F). (C) The testicular compartment shows rarely distributed seminiferous tubules containing CIS cells (arrowheads) and numerous immature Sertoli cells. Scale bars = 200 μ m (A), 40 μ m (B), 40 μ m (C).

Table 3. Karyotypes versus gonadal stoma and germ cell neoplastic lesion in patients with sex cord-derived tumors

Patient	Age	Karyotype	Left gonad (neoplastic lesions)	Right gonad (neoplastic lesion)
1	7 mo	46,XY, t(7;9)	Testis (GDA nests, CIS)	Testis (CIS)
2	7 y	46,XY	GDA-only GD	GDA-only GD
3	12 y	45,X/46,X,del(Y)(q12)	GDA-only GD	GDA-only GD
4	13 y	45,X/46,XY	Streak	Streak (GDA nests)
5	16 y	46,XY	GDA-only GD	GDA-only GD
6	17 y	46,XY	Streak	Streak (GDA nests)
7	19 y	46,XY	Testis	Testis (GDA nests)
8	3 mo	46,XY	Streak (MGCSCST)	Testis (CIS)
9	2 y	46,XY	Testis (MGCSCST, CIS)	Testis (MGCSCST, CIS)
10	3 y	45,X/46,XY	Testis (CIS)	Testis (MGCSCST, CIS)
11	5 y	46,XY	Testis (MGCSCST, CIS)	Streak

(Słowikowska-Hilczer, 2001; Słowikowska-Hilczer et al, 2001a) and were negative for the RNA-binding motif (RBM) protein of chromosome Y (Schreiber et al, 2002). The RBM protein is expressed in normal male germ cell nuclei of fetal (ie, from the second trimester of gestation), prepubertal, and adult testes (Elliott et al, 1997). The lack of RBM expression indicates impaired function of azoospermia factor-b (AZF-b), a region of the human Y chromosome long arm (Elliott et al, 1997). Although Lim et al (1998) have shown that only a minority (15%–20%) of XY patients with GD and sex reversal have a mutation in the sex-determining region of chromosome Y (SRY) to account for the phenotype, it cannot be excluded that the impaired function of AZF-b is a causative factor for the disturbances in testicular organogenesis that lead to GD.

The persistence of Müllerian derivatives in all our patients suggests that Sertoli cells of dysgenetic testes and bisexual gonads did not secrete anti-Müllerian hormone (AMH) during the fetal period, which might influence germ cell compartmentalization. However, immunohistochemical data from our 5 patients with 46,XY and GD, aged 2 to 5 years, and reported elsewhere (Schreiber et al, 2003), showed that AMH was present in Sertoli cells. This does not exclude the possibility that dysgenetic gonad secretes less AMH than normal fetal testes or that this AMH may have an aberrant action. The presence of Wolffian duct derivatives in almost equal frequencies in pure, mixed, and partial GD indicates that despite dysgenesis, fetal gonads secreted androgens in most cases. Wolffian ducts were less frequent in patients with gonadoblastoma-only GD and in those with bisexual gonads,

indicating disturbed androgen secretion or action in fetal life in both instances.

As would be expected, bisexual gonads developed in association with NSAS. Although the 46,XX karyotype was present in 2 patients, both had translocations of Yp onto the inactive X chromosome. Thus, these 2 patients were also considered as having NSAS. To our knowledge, besides the individual with ovotestis/ovary and 45,X/46,Xt(Yp;Xp) revealing CIS reported herein, CIS has never before been reported to occur in true hermaphroditism. It can be hypothesized that in true hermaphroditism ovarian secretion (primary follicles) counteracts the development of germ cell carcinoma through inhibition of the multiplication of primordial germ cells. That is, germ cells of the ovary stop proliferating and enter meiosis in fetal life. Furthermore, the incidence of germ cell tumors in the ovary occurs very infrequently (Holschneider and Berek, 2000).

In summary, it appears that, first, NSAS is not a prerequisite for the appearance of GD; GD is more frequently associated with 46,XY. Second, the spectrum of germ cell neoplastic lesions in GD is wider than was reported before and in addition to germ cell carcinoma, CIS, and gonadoblastoma nests, the spectrum also includes a bilateral tumor of gonadoblastoma-only GD and MGCSCST. Third, the incidence of neoplastic lesions is related more to the severity of the disturbances of gonadal organogenesis than it is to aberrations in sex chromosomes. And finally, less-disturbed testicular organogenesis predisposes more toward germ cell neoplastic lesions, which suggests that the testicular environment of a dys-

Table 4. Incidence of germ cell neoplastic lesions in relation to the presence of 46,XY and NSAS karyotypes

Karyotype	Germ cell tumor n (%)	Intratubular CIS n (%)	Sex cord tumor n (%)	Intratubular CIS+ sex cord tumor n (%)	Total n (%)
46,XY (n = 28)	1 (3.7)	12 (42.9)	4 (14.8)	3 (11.1)	20 (71.4)
NSAS* (n = 17)	0	3 (17.6)	1 (5.9)	2 (11.8)	6 (35.3)

* Two patients with 46,XX and bisexual gonads with proven translocation of the Yp fragment onto a late-replicating, inactive X chromosome (Kusz et al., 1999) were included.

Table 5. Laterality of germ cell neoplastic lesions in relation to GD type

Laterality of neoplastic lesions	Pure GD	Mixed GD	Partial GD	46,XY	NSAS
Unilateral	3 (100%)	9 (90%)	3 (30%)	11 (55%)	4 (80%)
Bilateral	0	1 (10%)	7 (70%)	9 (45%)	1 (20%)

genetic gonad plays an important role in germ cell neoplasia initiation, maintenance, or both.

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