Testicular Pathology in 46,XY Dysgenetic Male Pseudohermaphroditism: An Approach to Pathogenesis of Testis Cancer

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ABSTRACT: Eleven children with dysgenic male pseudohermaphroditism (DMP) and 18 boys with isolated penile hypospadias, all with 46,XY karyotype, were studied. Testicular dysgenesis was associated with significantly lower testosterone response to human chorionic gonadotropin (0.9 ± 0.2 ng/mL) than it was in hypospadias (3.3 ± 0.1 ng/mL), and with significantly higher mean serum follicle-stimulating hormone (FSH) levels (8.4 ± 2.3 IU/L vs 1.5 ± 0.3 IU/L). Gonadoblastoma, a tumor that arises from the sex cords, was found in more than ¼ of patients with DMP, whereas testicular carcinoma in situ (CIS) cells were present in all of these patients. Forty-two percent to 98% of CIS cells revealed an aneuploid pattern of nuclear DNA, indicating that most of them are neoplastic cells. In patients with hypospadias, CIS was not seen, and no other abnormalities were detected. In children with DMP, the percentage of tubules populated with germ cells was significantly lower than it was in those with hypospadias (48.3% ± 10.6% vs 92.4% ± 4.0%). The total number of germ cells (CIS cells + spermatogonia) did not differ significantly between the 2 groups, but the number of spermatogonia was significantly reduced in children with DMP (0.08 ± 0.05 vs 3.65 ± 0.2), suggesting impaired differentiation of gonocytes to spermatogonia. The following significant correlations were present with DMP: 1) the higher the seminiferous tubule cross-section area, the higher the number of CIS cells (r = 0.78); and 2) the higher the serum gonadotropin levels, the higher were tubular diameter (r = 0.93 for FSH and r = 0.75 for luteinizing hormone [LH]), area (r = 0.79 for FSH and r = 0.82 for LH), percentage of tubules populated with germ cells (r = 0.86 for FSH and r = 0.81 for LH), and number of CIS cells (r = 0.87 for FSH and r = 0.79 for LH). The results indicate that in intersex children with 46,XY karyotype, CIS occurs in dysgenetic testes in all cases and is frequently associated with gonadoblastoma. Impaired organogenesis of sex cords, relative inhibition of testosterone secretion, and the associated increased secretion of gonadotropins may create a milieu that induces or is favorable for the formation or maintenance of neoplastic lesions in dysgenetic testes early in childhood.

Key words: Gonadal dysgenesis, gonadotropins, testicular carcinoma in situ.


The earliest step of human spermatogenesis begins at the end of fetal life and consists of the differentiation of gonocytes, the fetal germ cells, into spermatogonia. The postnatal presence of abnormal germ cells similar in appearance to gonocytes is considered pathologic, and is termed testicular carcinoma in situ (CIS; Jørgensen et al, 1993). Gonocytes, testicular carcinoma, and CIS cells immunochemically express placental-like alkaline phosphatase (PLAP; Giwercman et al, 1991). It has been hypothesized and generally accepted that CIS arises from gonocytes during fetal development of the testis, remains silent during childhood, and transforms into overt tumors after the pubertal hormonal surge (Skakkebaek, 1972; Skakkebaek et al, 1984, 1987).

In fact, there are reports describing subfertile patients who developed germ cell tumors of the testis when treated with gonadotropins (Neoptolemos et al, 1981). Kotlar et al (1997) reported that a mutation in the luteinizing hormone (LH)/chorionic gonadotropin receptor occurs in human ovarian sex cord tumors. Martin et al (1998) reported a germ cell carcinoma that occurred in a 35-year-old man in whom gonadotropin-releasing hormone (GnRH)-independent, familiar, male-limited precocious puberty was present due to a constitutively activating mutation of the luteinizing hormone (LH)/chorionic gonadotropin receptor. Chen et al (1999) described 2 patients with 46,XY male pseudohermaphroditism who developed testicular germ cell tumors, one at age 17 and the other at age 31, in connection with a point mutation in the hormone-binding domain of the androgen receptor gene. These data may indicate the contribution of gonadotropins and testosterone to the pathogenesis of testicular cancer early in tumorigenesis.

In intersex individuals the prevalence of germ cell car-
cinoma is strikingly increased (Scully, 1970; Verp and Simpson, 1987). It has been suggested that in testes that are dysgenetic because of numerical aberrations in sex chromosomes, mostly 45,X/46,XY, and in cases of androgen insensitivity syndrome, CIS is present in childhood (Müller and Skakkebaek, 1984; Müller, 1985; Müller et al, 1999). No data are available regarding the occurrence of CIS in cases of testicular dysgenesis with the 46,XY karyotype.

We investigated the occurrence of CIS in dysgenetic testes of children with the 46,XY karyotype and correlated it with other seminiferous tubular pathologies and with serum levels of gonadotropins and testosterone.

**Materials and Methods**

**Patients**

Clinical material was collected in one center from 11 intersex children aged 8 months to 7 years (median age, 3 years). Criteria for the inclusion of patients in this study included the 46,XY karyotype, ambiguous external genitalia, and nonpalpable gonads. Gonads could not be detected by ultrasonography either in the labio-scrotum or in the inguinal canal. All 11 patients had female internal sex organs, revealed by ultrasonography and genitography. The patients were to have bilateral gonadectomy. During surgery, in 9 cases gonads were found bilaterally in the abdomen, in the position typical of an ovary, and unilaterally (second gonad absent) in 2 cases. Remnants of Wolffian ducts were not found.

Another group of 18 patients aged 1–6 years (median age, 4 years) with 46,XY karyotype, isolated penile hypospadias, and scrotal testes were also evaluated. Five boys with hypospadias (5–6 years old) underwent biopsy of the larger testis, on the basis of asymmetrical gonadal dimensions, which suggested pathology.

According to the Tanner (1962) pubertal development scale, all intersex and hypospadias patients were at stage I of secondary sexual characteristics (ie, the prepubertal stage).

**Hormonal Determinations**

Before surgery, blood levels of FSH and LH were determined. To determine testosterone levels, blood samples were taken twice, once before and once after 4 daily intramuscular injections of human chorionic gonadotropin (hCG; Pregnyl, N.V. Organon, The Netherlands) in a daily dose of 1500 IU/m² of body surface. Serum FSH and LH levels were estimated with a commercial immunoradiometric assay kit (Orion Diagnostica, Espoo, Finland), with intra-assay and interassay variations, respectively, of 1.0% to 3.9% and 5.6% to 6.7% for LH, and 1.5% to 2.7% and 2.0% to 8.6% for FSH. The sensitivity for both hormones was 0.1 IU/L.

A radioimmunoassay kit (Orion Diagnostica) with an intra-assay variation of 3.8% to 7.5% and an interassay variation of 4.8% to 7.0% was used to determine serum levels of testosterone. The testosterone assay had a sensitivity of 0.03 ng/mL (0.1 nmol/L). The cross-reactivity was 4.5% with dihydrotestosterone and less than 0.04% with other steroids.

The following reference hormonal values for normal boys in the first Tanner pubertal stage were taken from Cacciari et al (1982): FSH, 1.57 ± 0.19 IU/L; LH, 1.60 ± 0.38 IU/L; and testosterone, 0.23 ± 0.04 ng/mL.

**Histology and Immunohistochemistry**

Tissues were fixed in Bouins solution and embedded in paraffin. Each gonad of the intersex children and the biopsy material of boys with hypospadias were sectioned serially in their entirety. Several slides were stained with hematoxylin and eosin and examined histologically.

Five 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, which gives the cytoplasm in PLAP-positive cells a yellow-brownish staining. Paraﬃn-embedded sections of testicular seminoma that had been resected from an adult patient served as a positive control. For the negative control, the primary antibody was replaced with 0.05 M Tris-buffered saline in the same specimen. Immunohistochemistry was performed simultaneously (with the same kits of reagents) for all examined tissues.

**Morphometry**

Seminiferous tubule development was evaluated on the basis of tubular diameter and relative area of tubular cross-section. The relative area is a quotient of the areal fraction of tubules and their numerical density. Numerical density is the number of tubular cross-sections expressed per total area of gonadal cross-section (Gundersen et al, 1988). To evaluate the areal fraction of the seminiferous tubules the microscopic picture at 100× magnification was covered by a square lattice containing 441 intersections. The number of intersections (points) that appeared on the examined tubular cross-sections was counted by a predetermined and systematic movement across the grid over the entire tissue section. The areal fraction was calculated by dividing the number of points that hit the tubules by the number of points that hit the entire vision area at the same magnification. Whereas numerical density reports the number of tubule cross-sections per vision area at 100× magnification, the areal fraction
Table 1. Histopathology of the gonads and basal serum concentrations of FSH and LH in 11 intersex children (patients 1–11) and in 5 prepubertal boys with isolated penile hypospadias (patients 12–16), in whom testicular biopsy (*) was performed.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Gonads</th>
<th>Germ cell types</th>
<th>FSH (IU/L)</th>
<th>LH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. US</td>
<td>&lt;1</td>
<td>Streak</td>
<td>Te</td>
<td>CIS/G</td>
<td>19.8</td>
</tr>
<tr>
<td>2. MH</td>
<td>1</td>
<td>Absent</td>
<td>Te</td>
<td>CIS</td>
<td>3.0</td>
</tr>
<tr>
<td>3. MT</td>
<td>2</td>
<td>Te*</td>
<td>Te</td>
<td>CIS</td>
<td>13.9</td>
</tr>
<tr>
<td>4. AK</td>
<td>2</td>
<td>Te*, GDA</td>
<td>Te</td>
<td>CIS</td>
<td>12.3</td>
</tr>
<tr>
<td>5. MM</td>
<td>3</td>
<td>Te</td>
<td>Streak</td>
<td>CIS</td>
<td>2.7</td>
</tr>
<tr>
<td>6. MJ</td>
<td>3</td>
<td>Te*</td>
<td>Te</td>
<td>CIS</td>
<td>3.9</td>
</tr>
<tr>
<td>7. GW</td>
<td>3</td>
<td>Streak</td>
<td>Te</td>
<td>CIS + Sg</td>
<td>4.0</td>
</tr>
<tr>
<td>8. WP</td>
<td>4</td>
<td>Absent</td>
<td>Te</td>
<td>CIS + Sg</td>
<td>5.4</td>
</tr>
<tr>
<td>9. JK</td>
<td>5</td>
<td>Streak</td>
<td>Te, GDA</td>
<td>CIS</td>
<td>0.7</td>
</tr>
<tr>
<td>10. MW</td>
<td>6</td>
<td>Te</td>
<td>Streak</td>
<td>CIS</td>
<td>3.9</td>
</tr>
<tr>
<td>11. MS</td>
<td>7</td>
<td>GDA</td>
<td>GDA</td>
<td>CIS</td>
<td>23.0</td>
</tr>
<tr>
<td>12. KW</td>
<td>5</td>
<td>Te</td>
<td>Te*</td>
<td>Sg + Sc</td>
<td>0.8</td>
</tr>
<tr>
<td>13. MR</td>
<td>5</td>
<td>Te</td>
<td>Te*</td>
<td>Sg + Sc</td>
<td>1.8</td>
</tr>
<tr>
<td>14. WS</td>
<td>5</td>
<td>Te*</td>
<td>Te</td>
<td>Sg</td>
<td>2.8</td>
</tr>
<tr>
<td>15. AH</td>
<td>6</td>
<td>Te</td>
<td>Te*</td>
<td>Sg</td>
<td>1.0</td>
</tr>
<tr>
<td>16. RR</td>
<td>6</td>
<td>Te*</td>
<td>Te</td>
<td>Sg</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Investigated testis when both testes were present. Te indicates testis; CIS, carcinoma in situ cells; G, gonocytes; Sg, spermatogonia; Sc, spermatocytes; GDA, gonadoblastoma.

Figure 2. Result of immunostaining for the presence of PLAP in the testis of a 3-year-old intersex child. Note the coexistence of both PLAP-positive (yellow-brownish staining of cytoplasm) and PLAP-negative CIS cells. The nuclei of immature Sertoli cells are numerous in the vicinity of the basement membrane of the seminiferous tubule. Leydig cells are absent. Counterstained with hematoxylin. Bar = 20 μm.

Quantitative Analysis of Seminiferous Epithelium

A semiquantitative analysis of the seminiferous epithelium consisted of an estimation of the percentage of tubules populated by germ cells. Two hundred randomly selected tubular cross-sections were analyzed in each gonad.
Testicular Dysgenesis and Carcinoma In Situ

c) of diploid nuclei (2.0c) was calculated for at least 30 Sertoli before measurement. In each specimen, the mean DNA content CIS cells (Sandritter et al, 1966). Incomplete nuclei were ex-
which is approximately 120% of the median nuclear diameter of
sections were evaluated in each patient in sections stained with
sections stained with hematoxylin and eosin. Quantitative analysis of the seminiferous epithelium was performed in 20 cross-sections of seminiferous tubules in sections stained with hematoxylin and eosin for each patient. Germ cell types and Sertoli cells were recognized according to their morphological features (Holstein et al, 1988). In the testes of healthy prepubertal boys, the seminiferous epithelium contains immature Sertoli cells, type A-dark and type A-pale spermatagonia, and occasionally, spermatocytes (Gondos, 1975). A-dark spermatogonia have a homogenous, dark nucleus with a light vacuole. A-
pale spermatagonia have a light nucleus that contains fine chro-
matin, and a single or double nucleolus. The diameter of a sper-
matogonia nucleus is 5.7–7.1 μm.
The morphological criteria for recognizing CIS cells included a nucleus that was irregular in shape; irregular, coarse clumps of chromatin; and light and abundant cytoplasm (Holstein, 1987; Müller et al, 1987). Measurement of the nuclei of CIS cells re-
vealed that they were 7.5–13.1 μm in diameter.
Numbers of germ cells and Sertoli cells were expressed per
section of seminiferous tubule.

DNA Measurement

Gonads of 4 intersex children were selected to assess their con-
tent of nuclear DNA in germ cells that met the morphological
criteria of CIS cells. The patients represented different ages, and
an absence or coappearance of gonadoblastoma. Only CIS cells
located in seminiferous tubules outside of the gonadoblastoma
were investigated.
DNA content was determined by a densitometric measurement on Feulgen-stained sections (Jörgensen et al, 1995). The staining
procedure included hydrolysis in 5 M HCl at 22°C for 90 min-
utes followed by washing in distilled water, staining for 60 min-
utes in Schiff’s reagent, and finally, washing in a sulphite rinse
before mounting. Densitometry of nuclei was performed using the General Image Processing Software computer program (Luc-
ia, Niko, The Netherlands). An interference filter was used, which gave a monochromatic green light, with maximum light
transmittance at 560 nm. In order to minimize the risk of mea-
surement of cell nuclei, 12-μm-thick sections were used, which
is approximately 120% of the median nuclear diameter of
CIS cells (Sandritter et al, 1966). Incomplete nuclei were ex-
cluded by focusing on both the upper and the lower nuclear poles
before measurement. In each specimen, the mean DNA content
(c) of diploid nuclei (2.0c) was calculated for at least 30 Sertoli
cells nuclei (diploid standard cells), and the DNA content of CIS
cells was then expressed as multiples of the calculated haploid
value. The Sertoli cells were chosen for that purpose because they show very little proliferative activity (Nistal et al, 1982).

Statistical Analysis

Nonparametric analysis (Mann-Whitney U test) was applied for
comparison between groups after verification that values were not normally distributed. Correlations were examined using lin-
ear regression analysis. Mean values ± standard errors of the
mean (SEM) have been used to express group data. P < .05 was
considered significant. All statistical analyses were performed
using Statistica for Windows PL software, version 5.0 (Statsoft,
Kraków, Poland).

Results

Hormonal Determinations

In intersex children, excessive serum levels of FSH (19.8
and 23.0 IU/L) were present in 2 cases, and in 2 other
cases, they were elevated to a lesser degree (13.9 and 12.3
IU/L; Table 1). The mean serum FSH level was signifi-
cantly higher in the intersex group, whereas LH levels
did not significantly differ between the intersex and hy-
pospadias groups. A significantly lower mean serum level
of testosterone in response to hCG stimulation was pre-
sent in intersex children compared with the hypospadias
group (Table 2).

Histology, Immunohistochemistry, and DNA
Measurement

No pathology was seen in biopsy specimens of scrotal
testes in boys with isolated penile hypospadias. In 3 cases,
spermatogenesis was advanced up to spermatogonia, and
in the remaining 2 cases, spermatocytes were evident
(Figure 1). CIS cells were not present, and hormone levels
were in the normal prepubertal range.

Table 1 shows that in intersex children, gonads re-
vealed bilateral testicular structures in 3 cases; in 2 cases
the histological appearance was similar on both sides, and
in 1 case nests of gonadoblastoma were present unilater-
ally. One testis was selected for further examination, as
indicated in Table 1. Gonadoblastoma was also diagnosed in
2 other cases. In one patient, gonadoblastoma was lo-
cated within the testis in which a streak gonad was found
contralaterally, and in the another case, the whole gonads
had been replaced by gonadoblastoma bilaterally. Thus,
gonadoblastoma was diagnosed in more than 1/3 of inter-
sex children. Diagnosis of gonadoblastoma was based on
its characteristic appearance. It is composed of collections of
cellular nests surrounded by connective tissue stroma;
the nests are composed of a mixture of germ cells similar
to CIS cells and cells resembling immature Sertoli or
granulosa cells (Scully, 1970). Unilateral agenesis of go-
nads was present in 7 patients: in 5 patients, one testis

| Table 2. Mean ± SEM serum concentrations of sex hormones in 11 intersex children and in 18 boys with hypospadias |
|----------------|----------|----------|
| Hormone        | Intersex | Hypospadias |
| T (ng/mL)      | 0.12 ± 0.02 | 0.10 ± 0.01 |
| T + hCG (ng/mL)| 9.90 ± 0.23 | 3.27 ± 0.05 |
| FSH (IU/L)     | 8.42 ± 2.29 | 1.48 ± 0.27 |
| LH (IU/L)      | 2.60 ± 0.52 | 1.02 ± 0.27 |

* P < .05 vs hypospadias. T indicates basal testosterone; T + hCG, testosterone after hCG stimulation.
Seminiferous tubule development in 10 intersex children and in 5 boys with hypospadias†

<table>
<thead>
<tr>
<th></th>
<th>Intersex (mean ± SEM)</th>
<th>Hypospadias (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubules populated with germ cells (%)</td>
<td>48.33 ± 10.64</td>
<td>92.40 ± 4.03</td>
</tr>
<tr>
<td>Germ cell numbers:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIS positive for PLAP</td>
<td>1.38 ± 0.72 (41.3%)</td>
<td>0.00</td>
</tr>
<tr>
<td>CIS negative for PLAP</td>
<td>1.88 ± 0.61 (56.3%)</td>
<td>0.00</td>
</tr>
<tr>
<td>Spermatogonia</td>
<td>0.08± ± 0.05 (2.4%)</td>
<td>3.65 ± 0.20 (95.3%)</td>
</tr>
<tr>
<td>Spermatocytes</td>
<td>0.00</td>
<td>0.35 ± 0.21 (4.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>3.34 ± 1.09 (100.0%)</td>
<td>4.00 ± 0.28 (100.0%)</td>
</tr>
</tbody>
</table>

* P < .001 vs hypospadias (Mann-Whitney U test).
† Germ cell numbers are expressed per tubular cross-section (mean ± SEM).
CIS indicates carcinoma in situ cells; PLAP, placental-like alkaline phosphatase.

was accompanied by a contralateral streak gonad; and in 2 others, a second gonad was absent (Table 1).

High individual variations were found for the density of the distribution of seminiferous tubules within testicular sections in intersex children. Figure 1 demonstrates a rare distribution of seminiferous tubules with wide intertubular spaces, and rich with connective tissue. Only in 2 intersex children were spermatogonia present (patients GW and WP), and spermatocytes were seen in none (Table 1). Clinical findings and gonadal pathology led us to diagnose dysgenetic male pseudohermaphroditism (DMP) in all the examined intersex children.

Immunocytochemical staining showed that both PLAP-positive and PLAP-negative CIS cells (Figure 2) were present in most cases of DMP. In 3 patients (GW, WP, and MW) CIS cells were exclusively PLAP-negative. When associated with gonadoblastoma, CIS cells appeared both inside the tumor nests and within the seminiferous tubules. Table 3 indicates that the mean number of PLAP-positive CIS cells per tubule cross-section was slightly lower than the mean number of CIS cells with no expression of the PLAP antigen.

Densitometry of the nuclei of CIS cells revealed DNA content in the range of 1.96–6.78a. Table 4 shows that in the youngest examined individual the predominant (57.7%) DNA pattern of CIS cells was diploid (1.6–2.5c), whereas aneuploidy was demonstrated in 42.3% of cells. In contrast, CIS cells of the 3 remaining children aged 1 to 3 years revealed a high incidence (95.1%–97.6%) of aneuploidy, with triploidy and tetraploidy as the most frequent DNA content. Figure 3 demonstrates details in individual DNA pattern versus morphology of the seminiferous tubules in 2 children with DMP (8-month-old US and 2-year-old AK). In patient US, the appearance of nuclear chromatin was less irregular and less dense in comparison with all other DMP patients. In this patient, the DNA pattern of abnormal germ cells was predominantly diploid, which may indicate that among CIS cells, some unchanged gonocytes were present. In patient AK, some irregularities in seminiferous tubule cross-sections were noticeable, and may indicate aberrations in sex cord formation. In patients US and AK, CIS cells were both PLAP-positive and PLAP-negative.

Morphometry and Quantitative Analysis of Seminiferous Epithelium

The mean diameter of seminiferous tubules was 77.8 ± 9.2 μm in the DMP group and did not differ significantly in the hypospadias group (68.7 ± 6.0 μm). The relative area of seminiferous tubular cross-section also did not differ (7446.5 ± 1278.9 μm² in the DMP group and 7112.8 ± 868.5 μm² in the hypospadias group). The number of Sertoli cells per tubular cross-section was slightly higher in the DMP group than it was in the hypospadias group (57.2 ± 5.6 and 43.9 ± 3.9, respectively).

Table 3 shows that the mean percentage of tubules populated with germ cells was significantly lower in the DMP group than it was in the hypospadias group. The mean number of spermatogonia was significantly lower in the DMP group, whereas the total number of germ cells did not differ significantly. CIS cells contributed to about 97% of the total number of germ cells per tubular cross-section and were either PLAP-positive (41.3%) or PLAP-negative (56.3%).

Figure 3. Seminiferous tubule morphology with hematoxylin and eosin staining (upper panel) versus nuclear DNA distribution patterns (lower panel) of CIS cells in 8-month-old (A) and 2-year-old (B) intersex children. Note the presence of the nuclei of CIS cells (arrowheads). CIS cells were the only representations of germ cell lines. The nuclei of immature Sertoli cells (S) are numerous. In the 8-month-old patient, the appearance of nuclear chromatin of CIS cells is less irregular and dense, which may correspond to the prevalence of a diploid DNA pattern in the younger individual. Note the regular pattern of seminiferous tubule cross-section in the older child. This may indicate an aberration in sex cord development, which could be the early stage of gonadoblastoma. Bar = 20 μm.
Correlations

Significant correlations were present in the patients with DMP. Table 5 shows that the higher the serum FSH or LH levels, the higher were 1) tubular diameter, 2) its relative cross-section area, 3) the percentage of tubules populated with germ cells, and 4) total number of germ cells per tubular cross-section.

Discussion

According to the clinical definition (Berkovitz and Seehervong, 1998), DMP consists of bilateral or unilateral abnormal gonadal development, and the gonad on either side can vary from a streak gonad, to a partial dysgenetic testis, to an apparently normal testis. Development of female internal genitalia and female or ambiguous external genitalia is a consequence of defective testicular organogenesis, and diminished sex hormone secretion occurs in these patients (Grumbach and Conte, 1981; Rey et al., 1999). The patients diagnosed by us as having DMP follow the above criteria. Because Sertoli cells are the source of Müllerian inhibiting hormone (Danahoe et al., 1987), the presence of Müllerian duct derivatives indicates an impaired function of fetal Sertoli cells. According to Brown (1996) and Danahoe et al. (1987), the presence of Müllerian duct derivatives argues against the diagnosis of androgen insensitivity syndrome. In turn, the coexistence of Müllerian ducts with undescended testis argues against the diagnosis of 5α-reductase deficiency (Wilson et al., 1996). The genetic background of disturbances in testicular organogenesis is not completely solved. It has been shown that only 20% of cases of gonadal dysgenesis are explained by mutations in SRY or its flanking sequences (Lim et al., 1998).

In our clinical material no testicular pathology was present in isolated penile hypospadias. Previously, it has been shown that in this disturbance, peripheral androgen action is normal (Gearhart et al., 1988). Moreover, in isolated penile hypospadias, unlike in scrotal and perineal types, no changes in the pituitary-testicular axis were detected (Kula et al., 1981). This led us to assume that because of difficulties in obtaining control biopsy material from healthy boys, young patients with hypospadias can be considered as a reference group.

Using hypospadias as a reference group, a difference in age distribution between patients with DMP and the hypospadias group is noticeable. However, according to many developmental measures, the patients in both groups were prepubertal (Tanner stage I). Prepuberty is considered as the uniform, gonadally silent developmental
period (Gondos, 1975; Sizonenko et al, 1989). In comparison with the hypospadias group, seminiferous tubular abnormalities in our DMP patients consisted of 1) the presence of CIS cells in all cases, 2) the presence of gonadoblastoma in more than \( \frac{1}{4} \) of cases, 3) a near absence of spermatogonia, 4) a reduced number of seminiferous tubules populated with germ cells, and 5) impaired Leydig cell function as demonstrated by reduced serum testosterone levels in response to hCG stimulation.

Our densitometric probe showed that most CIS cells should be considered as neoplastic cells because of their hyperdiploidy. Hyperdiploidy is a characteristic feature of germ cell tumors (De Jong et al, 1990; Fischer et al, 1994). Previously, Müller and Skakkebaek (1981) demonstrated that DNA ploidy of CIS cells resembles the cells of adjacent invasive tumor, indicating that the aberration of DNA content occurs early during tumorigenesis. Our data support this concept and indicate that most CIS cells seen in children with DMP are neoplastically transformed. The prevalence of a diploid DNA pattern in the CIS cells of our 8-month-old patient may suggest that, in contrast to older individuals, the neoplastic transformation of gonocytes to CIS cells is less frequent at this age.

We report that in the testes of intersex children with 46,XY karyotype the incidence of CIS cells is unexpectedly high (100% of cases). This frequency has never been published before. Previously, CIS was found in 25% of patients with androgen insensitivity syndrome between the ages of 2 months to 19 years (Mueller and Skakkebaek, 1984), and in 4.7% of patients aged 1 month to 14 years with 46,XY karyotype who also had either androgen insensitivity or testicular steroidogenesis disorders, persistent Müllerian duct syndrome, gonadal dysgenesis, or true hermaphroditism (deducted from Rey et al, 1996). Until our study, no comparably large amount material of dysgenetic testes in individuals with 46,XY karyotype has been evaluated simultaneously. Our data may indicate that testicular dysgenesis particularly predisposes the appearance of CIS. Impaired Sertoli cell function could be involved; in particular, Kleisch et al (1998) have demonstrated that in adult men with unilateral testicular carcinoma, Sertoli cells associated with CIS in the contralateral testis underwent a process of dedifferentiation. They suggested that this dedifferentiation resulted in a loss of Sertoli cell function, led to cessation of spermatogenic activity, and to the appearance of CIS. We have shown here that the population of spermatogonia is almost absent in DMP, whereas CIS cells were numerous.

Our data show that more than half of CIS cells do not express PLAP antigen. PLAP-positive and PLAP-negative CIS cells were described previously (Hustin et al, 1987; Rajpert-De Meyts et al, 1996). The reason for this nonuniformity is unknown and it indicates that histological criteria for detection of CIS are also important.

In adult men, hypersecretion of FSH develops due to an inadequate feedback control of the pituitary, and is associated with spermatogenic injury (Bergmann et al,
Moderate increase in gonadotropin secretion can be present at prepuberty in boys with cryptorchidism, presumably as a result of gonadal inadequacy (Kula and Chilariski, 1987). Here we have shown that the mean level of FSH in the DMP group was higher than it was in the hypospadias group, indicating impaired Sertoli cell function. Paradoxically, serum levels of FSH and LH correlated not negatively, but positively, with the development of seminiferous tubules measured by diameter, relative area, and the percentage of tubules populated with germ cells.

One of the most important findings is that both FSH and LH were positively correlated with the number of CIS cells. It seems that gonadotropins might influence the germ cell compartment despite impaired Sertoli cell function. Yoon and Golimbu (1987), through immunocytology, localized human FSH binding in the luminal, basal, and interstitial areas of human seminiferous tubules that were affected by Sertoli barrier disorders, indicating that Sertoli cells may not be an exclusive target for FSH. We have previously shown that in adult men, elevated basal serum levels of FSH or GnRH-stimulated secretory reserves of FSH and LH were correlated with an increased number of spermatogonia (Kula, 1991; Kula and Slowikowska-Hilczer, 1997). Administration of FSH to adult men and nonhuman primates increases the number of spermatogonia (Alphen et al, 1998; Foresta et al, 1988). Subsequently, FSH receptor messenger RNA has been found in spermatogonia in normal human testes (Baccetti et al, 1998). Until now, no information has been available regarding the influence of FSH on the proliferation of gonocytes and CIS cells in men. In immature rats, purified human FSH stimulated mitotic activity of gonocytes before the formation of blood-testis barrier (ie, the functional maturation of Sertoli cells; Almiron and Chemes, 1988). All these data suggest that in men, both FSH and LH may participate in the creation of the numerical aspect of premeiotic germ cells, including CIS cells.

Chen et al (1999) demonstrated that a mutation of the hormone-binding domain of the androgen receptor gene in 2 patients with male pseudohermaphroditism with the 46,XY karyotype could be involved in early progression of germ cells toward oncogenesis in the dysgenetic testes. Our data showing a decreased response of testosterone to stimulation with hCG may indicate that decreased availability of testosterone is harmful to the development of the germinal cell compartment.

The presence of gonadoblastoma in the DMP group indicates an abnormal organogenesis of sex cords, the fetal precursors of seminiferous tubules. Gonadoblastoma is considered as an in situ testicular neoplastic lesion (Scully, 1981) that may give rise to seminoma and nonseminomatous germ cell tumors (Scully, 1970, 1981; Hart and Burgons, 1979; Talerman, 1980). Jørgensen et al (1997) have suggested that the germ cell tumors arise from CIS cells located inside gonadoblastoma nests. We show a high incidence of gonadoblastoma among patients with DMP (more than ¾ of cases), which is much higher than data deducted from Rey et al (1996), who gave a figure of 4.7% of cases with different causes of intersexuality.

Few risk factors have been proposed for testicular germ cell cancer. Perinatal exposure to sex hormones or “hormonal disruption” is proposed as one factor that influences the risk of malignant changes (Rajpert-DeMeys and Skakkebaek, 1993; Akre et al, 1996). The data presented here may suggest that developmental aberrations of testicular structure, decreased secretion of testosterone, and the associated increased secretion of gonadotropins may create a milieu that is favorable for the appearance of a preinvasive neoplastic lesion of germ cells in childhood.

In summary, it appears that cryptorchidism, disturbed organogenesis of sex cords, decreased Sertoli and Leydig cells function, the presence of CIS cells and their early malignant transformation, as well as underpopulation of seminiferous tubules with germ cells are constituents of testicular dysgenesis. Our data also show that in dysgenetic testes of patients with the 46,XY karyotype, the incidence of CIS cells is more frequent than it was believed to be before. We show that circulating gonadotropins positively correlated with the number of CIS cells, which may suggest that in testes with impaired organogenesis, gonadotropins may be involved in the formation or maintenance of germ cell pathology and, as a consequence, participate in the promotion of testis cancer.

References


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