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CLINICAL STUDY

Testicular function and physical outcome in young adult males diagnosed with idiopathic 46 XY disorders of sex development during childhood

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Abstract

Objective: There are few studies of outcome in male patients with undefined 46 XY disorder of sex development (DSD). We aimed to assess testicular function and clinical characteristics after puberty in men with idiopathic 46 XY DSD.

Design: We conducted a University Hospital-based observational follow-up study.

Methods: Nineteen patients with severe hypospadias associated with other signs of defective virilization, such as microphallus, cryptorchidism, and/or bifid scrotum, who were initially managed during childhood between 1988 and 1994, were evaluated at a median age of 17.6 (16.3; 17.8) years. Outcome measures included clinical findings and serum testosterone, FSH, LH, and inhibin B concentrations.

Results: Testicular function was normal in only five (26%) patients. Impaired testicular function was observed in 14 (74%) patients and was partial ($n=6$; 32%) or total ($n=8$; 42%), requiring testosterone treatment for the initial ($n=2$) or secondary ($n=6$) induction of puberty. Undescended testis (unilateral $n=3$, bilateral $n=2$) was found and surgically managed only in the 14 patients with testicular impairment. Testosterone treatment in early childhood greatly increased penis length in all patients, but persistent microphallus following surgical treatment was observed at the end of puberty in most patients, with no difference between patients with and without testicular dysfunction (penis length of 68 (60; 75) vs 65 (60; 65) mm; $P=0.42$). Half the patients presented an adult height more than 5 cm below their target height.

Conclusion: Men diagnosed with idiopathic 46 XY DSD during childhood are at high risk of testicular insufficiency and persistent micropenis, and this should be taken into account during the follow-up.

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Introduction

Considerable advances have been made over the last few decades towards an understanding of the complex developmental process underlying male sexual determination and differentiation and the management of patients with disorders of sex development (DSD) (1–4). 46 XY DSD is a heterogeneous disorder that may be associated with an increase in the risk of testicular dysfunction. Definitive etiological diagnosis is based on the clinical examination and the results of appropriate investigations. However, this disorder remains idiopathic in many cases (5–8).

There have been few studies of post-puberty outcome in male patients with undefined 46 XY DSD, and the studies carried out have included a mixture of known etiologies of DSD, with relatively small patient cohorts and often no investigation of testicular function carried out after puberty (7–9). Two recent studies have shown that boys and men with hypospadias associated with other features of defective virilization, such as microphallus, cryptorchidism, and bifid scrotum, have a higher risk of impaired testicular function than those with isolated hypospadias (6, 10). It is also a matter of concern that small penis size may persist into adulthood, becoming a major cause of dissatisfaction in these patients (8, 9, 11, 12).

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The aim of this study was to assess clinical characteristics and testicular function after puberty in men with 46 XY DSD of undefined etiology who had previously been medically managed and treated by reconstructive genital surgery in early childhood.

Patients

All patients born between 1988 and 1994 with idiopathic 46 XY DSD, raised as boys, who had completed puberty and reached adult height, and had been followed in our department, were studied.

The inclusion criterion for the study was proximal hypospadias associated with other signs of defective virilization, such as microphallus, cryptorchidism, and/or bifid scrotum.

In total, 29 patients with 46 XY DSD raised as boys were included in this study. Ten of these patients were lost to follow-up. The characteristics at the time of first evaluation of the participants ($n=19$) and non-participants ($n=10$) are shown in Table 1. The study population was representative of the entire population studied in our department, as shown by mean age at diagnosis, DSD severity assessed on the basis of the presence of microphallus, cryptorchidism, bifid scrotum, peak testosterone post-human chorionic gonadotropin (hCG) below 3 ng/ml, peak testosterone/dihydrotestosterone (DHT) post-hCG, and the presence or absence of a utriculovaginal pouch. Imaging studies showed that none of the patients presented Müllerian duct remnants.

Analysis of the initial clinical characteristics of the participants at the time of diagnosis in early childhood, at a median age of 0.3 (0.0; 2.1) years, showed that, in addition to proximal hypospadias, five patients (26%) had cryptorchidism (unilateral $n=3$, bilateral $n=2$) and ten patients (53%) had bifid scrotum. All but one patient had a small phallus, with a median phallus size for the whole population of -3 (-3.3; -2.4) SDS. Twelve of these patients presented severe microphallus. A blind utriculovaginal pouch with no uterus was found in seven (37%) patients. Ultrasound examination of the kidneys was normal in all patients. Additional malformations were observed in seven patients (37%). Three patients were born premature and two patients were small for gestational age. Participants were presumed to have partial gonadal dysgenesis ($n=7$; patients 5–8, 10, 12, and 19), 5α-reductase deficiency ($n=2$; patients 11 and 13), partial androgen insensitivity (patient 1), or undefined 46 XY DSD ($n=9$) according to the classification system used (6) (Table 2). However, molecular genetic analysis of the androgen receptor and steroid 5α-reductase gene found no mutations in any of the cases. In patients presumed to have partial gonadal dysgenesis, there were also no deleterious mutations in other genes known to be involved in testicular dysgenesis syndrome, such as those encoding NR5A1 (also called steroidogenic factor 1), WT1, and SOX9 (data not shown). All these 19 patients were therefore classified as having idiopathic 46 XY DSD.

Informed consent for the evaluation and treatment was obtained from the subjects or their parents. Written consent for the genetic study was obtained from parents and/or patients.

Methods

Physical evaluation data obtained in infancy or childhood and early in adulthood were assessed by one of us (J Léger). The appearance of external genitalia was described in terms of size and position of the testes when palpable, appearance of the scrotum, and hypospadias. Penis length was measured in a fully stretched flaccid state by placing a ruler along the dorsal surface of the penis and measuring from the pubis to the tip of the glans, while depressing the suprapubic fat pad, as described by Lee *et al.* (13). Microphallus was defined as a penis with a stretched flaccid length ≤ -2 SDS and severe microphallus was defined as a penis length more

Table 1 Characteristics of participants and non-participants at initial evaluation during childhood. Results are expressed as medians (25th and 75th percentile) or number (%).

	Participants ($n=19$)	Non participants ($n=10$)	*P* values
Chronological age (years)	0.3 (0.0; 2.1)	1.7 (0.1; 3.1)	0.20
Prematurity	3 (15%)	4 (40%)	0.32
Birth weight (SDS)	−0.8 (−1.4; −0.2)	−1.2 (−2.2; −0.4)	0.31
Associated malformations	7 (37%)	2 (20%)	0.43
Penis length (mm)	22 (17; 29)	28 (24; 30)	0.15
Microphallus	18 (95%)	7 (78%)	0.23
Bifid scrotum	10 (53%)	3 (30%)	0.24
Cryptorchidism			0.18
No	14 (74%)	5 (50%)	
Unilateral	3 (16%)	1 (10%)	
Bilateral	2 (10%)	4 (40%)	
Utriculovaginal pouch	7 (37%)	1 (10%)	0.10
Peak testosterone <3 ng/ml post-hCG	7 (37%)	1 (10%)	0.13
Testosterone/DHT	5.2 (2.3; 5.8)	6.7 (5.3; 8.1)	0.30

Table 2 Initial characteristics of the patients diagnosed with idiopathic 46 XY disorder of sex development during childhood, according to testicular function after puberty.

		Birth weight				Penis length					hCG stimulation				
TF after puberty/no.	**GA** (weeks)	g	SDS	**CA** (years)	**Associated malformations**	mm	SDS	**Cryptorchidism**	**Bifid scrotum**	**UV pouch**	Type*	Test. (ng/ml)	Test./ DHT	**Basal FSH** (IU/l)	**Basal LH** (IU/l)
Normal ($n=5$)															
1	35	2300	−0.4	0.3	LLD, triangular face, and motor impairment	20	−2.4	No	No	No	1500×3	9.8	5.4	2.3	2.3
2	40	2800	−1.7	8.6	LLD	30	−3.3	No	No	No	1500×3	4.2	5.6	0.7	0.1
3	40	2960	−1.3	0.5	No	17	−3.3	No	Yes	No	1500×3	5.4	4.5	2.2	0.9
4	38	3260	0.3	0.8	Syndactyly hand	20	−2.9	No	Yes	No	1500×3	3.9	5.3	0.5	0.5
5	40	3000	−1.3	0.8	No	27	−2.0	No	No	No	1500×3	2.6	5.5	0.2	0.1
Median (25–75 perc. %)	40 (38; 40)	2960 (2800; 3000)	−1.3 (−1.4; −0.5)	0.8 (0.4; 0.8)	3 (60%)	20 (20; 27)	−2.9 (−3.3; −2.4)	0 (0%)	2 (40%)	0 (0%)		4.2 (3.9; 5.4)	5.4 (5.3; 5.5)	0.7 (0.1; 2.2)	0.5 (0.1; 0.9)
PTD ($n=6$)															
6	41	3510	−0.2	3 days	NF and BWS	25	−2.5	No	Yes	Yes	1500×3	1.3	1.3	0.6	0.4
7	38	3000	−0.3	3 days	No	26	−2.3	No	Yes	No	1500×3	1.5	–	–	–
8	41	3000	−1.4	8.4	No	46	−1.7	Right	No	No	1500×6	1.2	–	0.6	0.1
9	40	3520	−0.0	1 day	No	22	−3.3	No	Yes	Yes	1500×3	3.6	2.3	0.7	0.5
10	38	2630	−1.2	2.1	Tetralogy of Fallot	29	−2.4	Bilateral	No	No	1500×3	1.9	1.2	0.6	0.5
11	39	2690	−1.6	10.0	IVC	30	−3.1	No	No	No	1500×3	3.3	66.0	0.8	0.5
Median (25–75 perc. %)	39.5 (38; 41)	3000 (2690; 3510)	−0.8 (−1.4; −0.2)	1.7 (0.0; 8.4)	3 (50%)	27.5 (25; 30)	−2.4 (−3.1; −2.3)	2 (33%)	3 (50%)	2 (33%)		1.7 (1.3; 3.3)	6.7 (1.8; 38.5)	0.6 (0.6; 0.7)	0.5 (0.4; 0.5)
TTD ($n=8$)															
12	33	1290	−2.6	0.7	No	20	−2.9	No	Yes	Yes	1500×3	2.6	5.8	0.5	0.5
13	39	3100	−0.6	9.5	No	30	−3.3	Left	No	Yes	1500×3	6.9	34.3	0.7	0.1
14	30	910	−2.7	1 day	No	13	−3.0	No	Yes	Yes	–	–	–	2.5	3.4
15	37	2570	−0.8	2 days	Polydactyly	18	−4.3	No	Yes	No	1500×3	4.7	2.9	0.5	0.5
16	39	3510	0.4	3 days	No	13	−5.5	No	Yes	No	1500×3	4.3	1.9	0.5	0.5
17	39	2860	−1.1	0.2	No	15	−5.0	Left	No	Yes	1500×3	3.6	4.8	2.6	2.8
18	38	3100	−0.1	2 days	No	27	−2.0	No	Yes	No	1500×3	3.3	–	1.0	3.1
19	39	3140	−0.5	0.1	No	8	−6.8	Bilateral	No	Yes	1500×7	0.1	1.0	8.9	0.5
Median (25–75 perc. %)	38.5 (35; 39)	2980 (1930; 3120)	−0.7 (1.9; −0.3)	0.0 (0.0; 0.5)	1 (12.5%)	16.5 (13; 23)	−3.8 (−5.2; −2.9)	3 (37%)	5 (63%)	5 (63%)		3.6 (2.6; 4.7)	3.9 (1.9; 5.8)	0.9 (0.5; 2.6)	0.5 (0.5; 3)

Test., testosterone; TF, testicular function; PTD, partial testicular dysfunction; TTD, total testicular dysfunction; GA, gestational age; CA, chronological age; UV pouch; utriculo vaginal pouch; perc., percentile; LLD, limb length discrepancy; NF, neurofibromatosis; BWS, Beckwith – Wiedemann Syndrome; IVC, interventricular communication; *, units × number of infections.

than 2.5 SD below the mean value. A complete physical examination, testicular and adrenal function evaluation, and karyotype determination were carried out for each patient at diagnosis. Patients with microphallus were treated with testosterone (injection of 100 mg/m^2 per 2 weeks for 2 months) during early childhood and all patients underwent reconstructive genital surgery for hypospadias. The timing and number of urethroplasty interventions were obtained from surgical records, together with data for the descent of the testes, if required. The presence of urethral fistula, urethral stenosis, or voiding dysfunction was recorded.

Internal genitalia were assessed by ultrasound scan and genitography. Target height was calculated from midparental height (14).

Hormonal evaluation at the time of diagnosis included basal LH, FSH, and testosterone concentrations. A standard ACTH stimulation test gave normal results for all patients, excluding the possibility of a steroidogenic pathway defect. Testosterone and DHT concentrations were measured after hCG stimulation (1500 IU hCG every other day for six (in infancy) or 12 days (after the age of 4 months)). A testosterone response to hCG stimulation of <3 ng/ml was considered insufficient and similar to partial gonadal dysgenesis (15). In patients with an impaired testosterone response to the hCG test, the testosterone/delta-4 ratio was normal (>0.5), excluding 17β-hydroxysteroid dehydrogenase deficiency. We evaluated 5α reductase activity from the testosterone/DHT ratio after hCG stimulation. A high testosterone/DHT ratio was considered predictive of 5α-reductase deficiency and a high basal testosterone concentration after hCG stimulation associated with high basal LH concentration was considered predictive of partial androgen insensitivity syndrome (6, 16, 17).

Testicular function evaluation was repeated at the time, or during puberty, if deemed necessary based on clinical examination of the progression of puberty, and at the end of puberty when the patient had reached adult height (height velocity ≤ 1 cm/year). Testicular function was assessed by determining basal serum testosterone, inhibin B, LH, and FSH concentrations. Patients with testosterone levels below 3 ng/ml and/or LH concentrations above 10 IU/l at the last evaluation and those receiving androgen replacement therapy were considered to have Leydig cell impairment. FSH concentrations above 10 IU/l or inhibin B concentrations below 60 pg/ml were considered to reflect impairment of the Sertoli cells and/or spermatogenesis. After puberty, a single testis volume of 12 ml was considered to be the lower limit of the normal range (18).

Hormone assays

Testosterone concentration was determined by RIA Testosterone Direct (Immunotech-BeckmanCoulter, Roissy, France). The intra- and inter-assay coefficients of variation (CV) were $<9.5\%$. The normal range of testosterone concentration for young men obtained in the laboratory was 3–12 ng/ml (10–35 nmol/l). FSH and LH concentrations were determined by fully automated two-site chemiluminescence immunoassays (Advia Centaur CP, Siemens Healthcare Diagnostics, Saint-Denis, France). The intra- and inter-assay CV were $<5\%$. For FSH and LH, the reference ranges are 1–8.6 and 1.5–7.9 IU/l respectively.

Inhibin B concentration was determined in a solid-phase sandwich assay with Oxford Bioinnovation reagents (Diagnostic Systems Laboratories, distributed by Beckman-Coulter, Villepinte, France). The intra- and inter-assay CV were 5.7 and 12% respectively. The normal range of inhibin B concentration for young men obtained in the laboratory is 110–330 pg/ml.

Molecular analysis

Genomic DNA was isolated from leukocytes prepared from the patients' blood samples, by standard procedures. PCR primers, amplification conditions and androgen receptor, steroid 5α-reductase, NR5A1, and *WT1* gene sequences have been described elsewhere (19–22). Exons 1–2 of the *SOX9* gene were amplified by PCR with the following primers: Ex1F, 5′-CGCCTTCCTAAGTGCTCGCC-3′; Ex1R, 5′-ACTCTGAGCCACAGTTACAC-3′; Ex2F, 5′-TGTGCAGAGGAAGCCGAGTG-3′; and Ex2R, 5′-AAGAATCTCCCAGGCGGAGG-3′. Two set of primers were used to amplify exon 3 of the *SOX9* gene: Ex3aF, 5′-CCCGGAGGGTGCCTAAGACTA-3′; Ex3aR, 5′-CTGCTGCTCGCTGTAGTGG-3′; Ex3bF, 5′-TGTGGATGTCCAAGCAGCAG-3′; and Ex3bR, 5′-TTAGGATCATCTCGGCCATC-3′. PCR was performed with *Taq* PCR Master Mix (Qiagen). PCR products were sequenced automatically with the ABI Prism BigDye Terminator sequencing kit on an ABI 3130 genetic analyzer, according to the manufacturer's instructions (Applied Biosystems, Courtaboeuf, France). All molecular analyses were performed in the same laboratory (F Paris and C Sultan).

Statistical analysis

Results are expressed as numerical values (percentages) for categorical variables and as medians (25th–75th percentile) for continuous variables. Comparisons of the characteristics of different groups of patients were based on chi-square tests or Fisher's exact tests for categorical variables, Wilcoxon tests for continuous variables (when comparing two groups), or Kruskal–Wallis ANOVA (when comparing more than two groups) as appropriate. All tests were two tailed with $P<0.05$ considered significant. Statistical analyses were performed using the SAS 9.1 (SAS, Inc., Cary, NC, USA) software package for PC.

Table 3 Characteristics of the patients after or close to the completion of puberty, according to testicular function.

TF/no.	CA (y)	AH (cm)	TH (cm)	AH−TH (cm)	Puberty	Tanner stage	Penis length (mm)	TV (ml)		Test. (ng/ml)	Inhibin B (pg/ml)	FSH (IU/l)	LH (IU/l)
								Right	Left				
Normal (n=5)													
1	17.4	151	163	−12	Spont.	5	65	15	15	5.4	83	4.7	5.0
2	16.3	170	173	−3	Spont.	5	60	25	25	7.4	199	0.4	1.7
3	15.5	162	169	−7	Spont.	4	55	15	15	5.1	69	4.7	5.8
4	14.5	170	179	−9	Spont.	5	65	25	25	4.0	90	3.6	1.6
5	16.9	172	179	−7	Spont.	5	65	25	25	5.4	89	3.8	2.9
Median	16.3	170	173	−7			65	25	25	5.4	89	3.8	2.9
(25–75 perc.)	(15.5; 16.9)	(162; 170)	(169; 179)	(−9; −7)			(60; 65)	(15; 25)	(15; 25)	(5.1; 5.4)	(83; 90)	(3.6; 4.7)	(1.7; 5.0)
PTD (n=6)													
6	16.8	186	175	+11	Spont.	5	60	25	25	2.7	69	2.1	3.5
7	15.2	173	175	−2	Spont.	4	70	20	15	2.4	101	2.1	2.9
8	20.3	176	172	+4	Spont.	5	80	25	20	4.6	17	7.7	5.6
9	16.2	183	175	+8	Spont.	5	85	20	25	6.2	10	13.9	2.5
10	20.8	166	174	−8	Spont.	5	75	20	25	4.9	35	7.8	5.6
11	16.6	168	–	–	Spont.	4	55	20	20	3.8	16	4.5	1.4
Median	18.4	174	175	4 (−2; 8)			72	20	20	4.2	26	6.1	3.2
(25–75 perc.)	(16.2; 20.8)	(168; 183)	(174; 175)				(60; 80)	(15; 25)	(15; 20)	(2.7; 4.9)	(16; 69)	(2.1; 7.8)	(2.5; 5.6)
TTD (n=8)													
12	17.6	158	166	−8	S Ind	5	40	12	12	TRT	10	0.4	0.1
13	17.7	163	165	−2	S Ind	5	75	30	–	TRT	56	3.6	1.7
14	16.5	170	–	–	S Ind	5	70	15	15	TRT	56	16	1.6
15	17.3	168	174	−6	S Ind	5	65	12	12	TRT	38	5.0	2.1
16	17.0	163	176	−13	S Ind	5	70	20	15	TRT	25	9.4	0.8
17	17.7	162	–	–	S Ind	5	55	2	1	TRT	0.0	78.2	33.4
18	17.9	183	174	+9	P Ind	5	60	20	20	TRT	41	5.3	2.6
19	17.8	166	163	+3	P Ind	5	60	–	–	TRT	0.0	0.4	0.1
Median	17.6	164	170	−4 (−8; 3)			62	13	12		31	5.1	1.6
(25–75 perc.)	(17.1; 17.5)	(162; 169)	(165; 174)				(57; 70)	(7; 20)	(0.5; 15)		(5; 48)	(2.0; 12.7)	(0.4; 2.3)

PTD, partial testicular dysfunction; TTD, total testicular dysfunction; TF, testicular function; TV, testicular volume; CA, chronological age; AH, adult heigth; TH, target height; Spont, spontaneous; S Ind, secondary induction; p ind, primary induction; Test., testosterone; perc., percentile; TRT, testosterone replacement therapy.

Results

The clinical characteristics of the patients are given as a function of testicular function at initial (Table 2) and most recent evaluations (Table 3).

At the time of the study, after the completion of puberty at a median age of 17.6 (16.3; 17.8) years, five (26%) patients had normal testicular function, with the spontaneous occurrence of puberty, which proceeded at a normal pace. None of these patients had cryptorchidism.

Six (32%) patients had partial testicular dysfunction. Puberty occurred spontaneously in all these cases, but, at the time of the study, two patients had low testosterone levels similar to impaired endocrine testicular function and four had normal serum testosterone levels but low serum inhibin B concentrations of 10–35 pg/ml, with slightly high basal FSH values, similar to impaired exocrine testicular function. Two of these patients had undergone surgery for unilateral ($n=1$) or bilateral ($n=1$) cryptorchidism.

In the remaining eight patients (42%), primary or secondary sex steroid treatment was required at the time of puberty for the induction and completion of puberty respectively. All these patients are currently on androgen replacement therapy for total testicular dysfunction. Serum inhibin B concentration ranged from 0 to 56 pg/ml. Three patients had undergone surgery for unilateral ($n=2$) or bilateral ($n=1$) cryptorchidism.

Penis length

Testosterone treatment during childhood, initiated at the age of 0.4 (0.0; 1.3) years, induced a dramatic increase in penis length in all patients, with a similar gain of penis length in patients with and without testicular dysfunction (+2.0 (1.0; 4.0) vs 1.6 (1.1; 1.9) SDS; $P=0.54$ respectively; Fig. 1). Nevertheless, at the end of puberty, after surgical treatment and with androgen therapy when required, persistent microphallus was observed in most patients, with a penis length of 65 (60; 65), 72 (60; 80), and 62 (57; 70) mm for patients with normal, partial, and total testicular dysfunction, respectively, with no significant difference between groups ($P=0.43$; Fig. 2).

Testicular volume

Testicular volume was within the normal range in patients with normal testicular function or partial testicular dysfunction. However, low testicular volumes were identified in five of the eight patients with total testicular dysfunction (Table 3). Three of these patients were previously found to have unilateral ($n=2$) or bilateral ($n=1$) undescended testis.

Adult height (or near-adult height)

Adult height (or near-adult height) was more than 5 cm below target height in eight (50%) of the 16 patients for whom data were available (Table 3).

Surgical results

As shown in Table 4, median age at initial surgery was 3 (2.7; 4.5) years for hypospadias ($n=19$) and 7.8 (3.0; 8.9) years for cryptorchidism ($n=5$). The patients underwent a median number of two procedures for hypospadias. The most common complications were urethral fistula ($n=9$ patients) and urethral stenosis ($n=3$ patients). Six patients had an additional procedure for penoscrotal transposition ($n=3$), buried penis ($n=1$), or recurrent ventral curvature ($n=1$). During the last evaluation, all but two of the patients had a straight penis with an apical or glandular meatus, one patient had a persistent fistula, and two patients were dissatisfied due to wide spraying of the urine stream.

Discussion

The results of this study extend our knowledge of testicular function and outcome after puberty in young adult patients managed since early childhood for severe hypospadias associated with other signs of defective virilization. In this group of 19 patients with undefined 46 XY DSD, only five patients (26%) were found to have normal testicular function at the end of puberty. Among these 19 patients, those with at least one undescended testis at initial evaluation were at higher risk of impaired exocrine or global testicular function early in

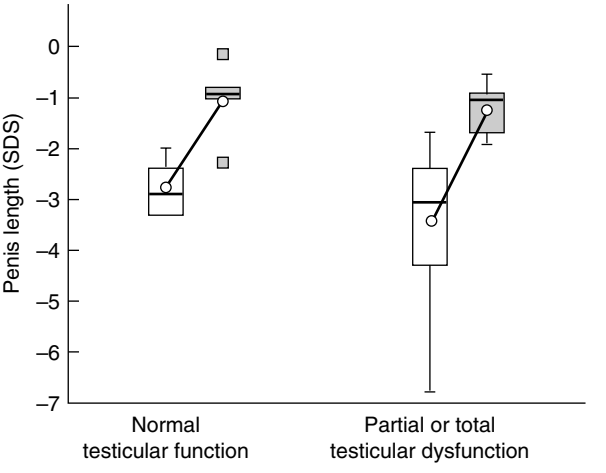

Figure 1 Box and whiskers plot of penis length (SDS) before (white) and after (gray) testosterone therapy initiated at the age of 0.4 (0.0; 1.3) years, in patients with 46 XY idiopathic disorder of sex development, according to testicular function after puberty. The upper and lower boundaries of the box indicate the 75th and 25th percentiles respectively. The whiskers show the range of the data.

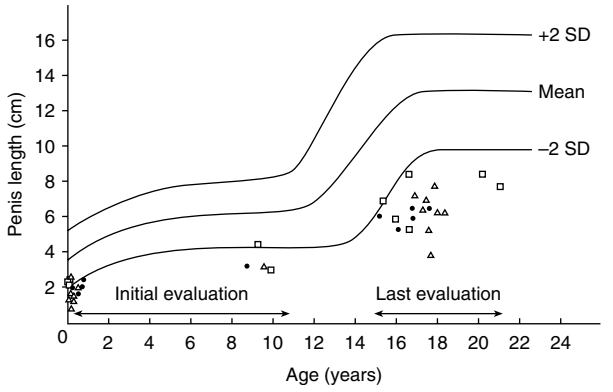

Figure 2 Penis length (cm) in patients with 46 XY idiopathic disorder of sex development, at the initial evaluation during childhood and after or near the completion of puberty, at a median age of 17.6 (16.3; 17.8) years, according to testicular function in early adulthood: normal (black dots), partial (white squares), or total (white triangles) testicular dysfunction. The normal values were obtained from the data of Lee *et al.* (13).

adulthood, as five (36%) of the 14 patients with some testicular function impairment at their most recent evaluation initially had one ($n=3$) or two ($n=2$) undescended testes, whereas all patients with normal testicular function after puberty were born with normally descended testes. Testicular dysfunction also occurred in patients without undescended testes. These findings are similar to previous data showing an impairment of Leydig and Sertoli cell function in 57% of boys with hypospadias, associated with short penis length and/or cryptorchidism (6). Our results are also similar to the concept of testicular dysgenesis syndrome, suggesting that impaired development of the fetal testes may lead to an increase in the risk of hypospadias and other signs of abnormal sex development (23, 24). However, this does not exclude the possibility that cryptorchidism may itself also have contributed to the impairment of testicular function, in at least some cases. We did not investigate semen quality, but these results suggest that these patients may have a low semen quality and a high risk of poor fertility later in life (25).

Furthermore, despite a significant increase in penis length following testosterone treatment in early childhood, penis length remained significantly lower than the reference values for young adults after surgical treatment and androgen therapy. This finding is similar to previous reports for young adults with microphallus associated with DSD, regardless of its etiology (8, 9, 12), and with the only other longitudinal study analyzing the efficacy of testosterone treatment during early childhood for increasing adult penis size in these patients (11). Intramuscular testosterone treatment has been shown to increase penis length effectively in prepubertal boys with hypospadias (11, 26, 27). We previously compared the effect of this treatment between two groups of children with similarly small penises, with and without hypospadias, and showed that the increase in penis length after treatment was smaller in patients with hypospadias than in those without hypospadias (26). Testosterone has also been shown to restore penis length to values between the mean and −2 SDS in most adult patients with congenital micropenis without male differentiation defects (28). This suggests that there may be differences in pathophysiological aspects of penile development between patients with microphallus associated with hypospadias and those with isolated micropenis.

The role of androgens in the control of penis development is unclear. It has been shown that adult penis size in rats is critically dependent on androgen action during the fetal masculinization programming window, during which androgens must act to ensure correct subsequent development. Moreover, postnatal testosterone treatment does not increase the size of the adult penis beyond its 'predetermined' length, though growth toward this maximum is advanced by peripubertal testosterone treatment (29, 30).

Another matter of concern in the population studied here is the high frequency of patients not reaching their target height; to our knowledge, this aspect has not been characterized in detail before. The association between hypospadias and fetal growth impairment and/or a syndromic phenotype, as observed in some of our patients, is well known (4, 31). It remains unclear whether this association indicates the existence of a causal relationship or a shared pathogenic factor (32). Some of the etiologies of testicular dysgenesis syndrome have been linked to genetic determinants, but complex interactions between inherited genetic susceptibility, epigenetic developmental changes, prenatal exposure, and lifestyle factors are also thought to occur (1, 24). It remains unclear whether the endocrine dysfunction alone or the low birth weight (associated or unassociated with the syndromic phenotype) observed in some of our patients attributable to birth at a younger gestational age or the baby being small for gestational age contributed to the statural growth deficit. However, we were unable to explore these hypotheses in this study, due to the small size of the cohort of patients.

Table 4 Surgical management according to testicular function. Data are presented as median (25–75 percentile).

	Normal ($n=5$)	**PTD** ($n=6$)	**TTD** ($n=8$)
CA at initial surgery for HS (years)	3.2 (3; 4.1)	4.3 (4; 6)	2.7 (2.6; 2.8)
Fistula	2	3	4
No. of repeat procedures for HS/patient	1 (0; 1)	2 (2; 2)	1.5 (1; 2)
Surgery for bifid scrotum	1 (14%)	1 (25%)	4 (44%)
CA at initial surgery for cryptorchidism (years)	–	5.8 (2.8; 8.8)	7.7 (3.0; 15.2)

PTD, partial testicular dysfunction; TTD, total testicular dysfunction; CA, chronological age; HS, hypospadias.

In this study, we were able to follow 66% of a well-defined population of patients with idiopathic 46 XY DSD to adult height. The main limitations of our study were the observational nature of data collection and the difficulties identifying partial testicular dysfunction (at this age). We tried to avoid this problem by systematically analyzing both tubular (Sertoli cells) and interstitial (Leydig cells) serum markers of testicular function. Despite the relatively small size of the sample studied, testicular insufficiency was found in a significant proportion of this young adult population. However, we cannot rule out the possibility that some of the patients not followed up had satisfactory results without testicular dysfunction or that some had a poor outcome with a normal pace of puberty and growth. These factors may have resulted in an overestimation of effect size in our data. However, the equal representation of patients with different types of phenotypic 46 XY DSD at initial presentation limits the likelihood of such a bias. Another important consideration is the underlying cause of the DSD. This study highlights the difficulties involved in establishing the etiological diagnosis. Within our population, we found no defects in genes involved in the production of active androgen metabolites or their action or in genes among those most frequently considered to account for variable degrees of gonadal dysgenesis in humans, such as those encoding *NR5A1, WT1*, and *SOX9*, highlighting the rarity of pathological abnormalities in the genes most frequently identified as involved in DSD. This suggests that other genetic and/or environmental factors are probably involved in the pathogenesis of this heterogeneous condition with considerable phenotypic variability. Recent technological advances may also make it easier to identify novel genetic markers for DSD in the future (33). The finding that testicular function is clearly defective in most cases provides a strong argument in favor of the hypothesis of a pre-existing testicular dysfunction in the fetus leading to defective testosterone production *in utero*. By contrast, in the small proportion of patients with apparently normal postnatal testosterone function, markers of defective testosterone action should be sought, because this is the most likely cause of the DSD in these patients.

In conclusion, this study provides evidence of impaired testicular function, small penis size, and adult height below target height in a large proportion of patients with 46 XY DSD of undefined etiology studied after the completion of puberty. These results have important clinical implications. These patients should be offered investigations of testicular function and follow-up at the time of puberty and in early adulthood. This is particularly important for individuals with low testicular volume, but even those with testes of normal size were found to have a higher risk of impaired testicular function. Patients and families should also receive education and support from a multidisciplinary team (approach) concerning long-term cosmetic outcomes, possibilities for reproduction, and the need for long-term sex hormone replacement in at least some cases. Moreover, the possibility of a deterioration of testicular function over time in a subset of patients should be considered and careful long-term follow-up of this population is therefore warranted. More population-based studies on larger groups of patients should be carried out in the future. Studies should also investigate the unknown potential risk of testicular cancer in patients with testicular dysgenesis in adulthood and long-term psychosexual development in this group.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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