

Approach to the Patient

Approach to the Virilizing Girl at Puberty

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Abbreviations: 17OHP, 17-hydroxyprogesterone; AMH, anti-Müllerian hormone; CAH, congenital adrenal hyperplasia; DHEA-S, dehydroepiandrosterone sulfate; DMRT1, Doublesex and Mab-3 Related Transcription factor 1; DSD, disorder/difference of sex development; E2, estradiol; FISH, fluorescent in situ hybridization; GCC, germ cell cancer; GCT, germ cell tumor; POR, cytochrome P450 oxidoreductase; SDS, standard deviation score; TS, Turner syndrome.

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Abstract

Virilization is the medical term for describing a female who develops characteristics associated with male hormones (androgens) at any age, or when a newborn girl shows signs of prenatal male hormone exposure at birth. In girls, androgen levels are low during pregnancy and childhood. A first physiologic rise of adrenal androgens is observed at the age of 6 to 8 years and reflects functional activation of the zona reticularis of the adrenal cortex at adrenarche, manifesting clinically with first pubic and axillary hairs. Early adrenarche is known as “premature adrenarche.” It is mostly idiopathic and of uncertain pathologic relevance but requires the exclusion of other causes of androgen excess (eg, nonclassic congenital adrenal hyperplasia) that might exacerbate clinically into virilization. The second modest physiologic increase of circulating androgens occurs then during pubertal development, which reflects the activation of ovarian steroidogenesis contributing to the peripheral androgen pool. However, at puberty initiation (and beyond), ovarian steroidogenesis is normally devoted to estrogen production for the development of secondary female bodily characteristics (eg, breast development). Serum total testosterone in a young adult woman is therefore about 10- to 20-fold lower than in a young man, whereas midcycle estradiol is about 10- to 20-fold higher. But if androgen production starts too early, progresses rapidly, and in marked excess (usually more than 3 to 5 times above normal), females will manifest with signs of virilization such as masculine habitus, deepening of the voice, severe acne, excessive facial and (male typical) body hair, clitoromegaly, and increased muscle development. Several medical conditions may cause virilization in girls and women, including androgen-producing tumors

of the ovaries or adrenal cortex, (non)classical congenital adrenal hyperplasia and, more rarely, other disorders (also referred to as differences) of sex development (DSD). The purpose of this article is to describe the clinical approach to the girl with virilization at puberty, focusing on diagnostic challenges. The review is written from the perspective of the case of an 11.5-year-old girl who was referred to our clinic for progressive, rapid onset clitoromegaly, and was then diagnosed with a complex genetic form of DSD that led to abnormal testosterone production from a dysgenetic gonad at onset of puberty. Her genetic workup revealed a unique translocation of an abnormal duplicated Y-chromosome to a deleted chromosome 9, including the *Doublesex and Mab-3 Related Transcription factor 1 (DMRT1)* gene.

Learning Objectives

Identify the precise pathophysiologic mechanisms leading to virilization in girls at puberty considering that virilization at puberty may be the first manifestation of an endocrine active tumor or a disorder/difference of sex development (DSD) that remained undiagnosed before and may be life-threatening. Of the DSDs, nonclassical congenital adrenal hyperplasia occurs most often.

Provide a step-by-step diagnostic workup plan including repeated and expanded biochemical and genetic tests to solve complex cases.

Manage clinical care of a girl virilizing at puberty using an interdisciplinary team approach. Care for complex cases of DSD manifesting at puberty, such as the presented girl with a Turner syndrome-like phenotype and virilization resulting from a complex genetic variation.

Key Words: virilization, androgen excess, disorders/differences of sex development (DSD), endocrine active tumors, genetic disorders of androgen excess

An 11.5-year-old girl was referred to our center for progressive clitoromegaly for 6 months. Her medical history revealed prematurity of 36 weeks' gestation with a birth weight of 2920 g (−0.70 standard deviation score [SDS]) and length of 45.5 cm (−1.85 SDS). At birth, typical female external genitalia were noted without any signs of virilization (eg, no clitoromegaly). The child was assigned and raised as female. She was followed by a general pediatrician who noted a mild delay in psychomotor development for unknown reasons, which did not prompt further neurodevelopmental and genetic workup. The girl had pubarche and thelarche at around age 10 years. At presentation aged 11.5 years, physical examination revealed a height of 139.4 cm (−1.12 SDS), a weight of 43.6 lb (0.47 SDS), and body mass index of 22.4 kg/m² (1.59 SDS), indicating overweight. Blood pressure was normal. She presented no syndromic features (eg, no typical signs for Turner syndrome [TS] or hypercortisolism). She showed a normal cardiopulmonary and abdominal examination; no intra-abdominal or inguinal masses were palpable. External genitalia inspection revealed a marked clitoromegaly of 3.5 × 1.5 cm in size but was otherwise typical female. No other signs of virilization such as voice deepening or severe

acne were noted. Pubertal stage according to Tanner was pubic hair V, breast II, axillary hair II. In addition, rich bodily hair was noted consistent with hypertrichosis, but a Ferriman and Gallwey score was not assessed.

Background

Virilization at puberty is a rare disease manifestation of severe androgen excess, most often occurring in girls who have an undiagnosed underlying genetic condition or an acquired disorder affecting the adrenal glands or gonads. By contrast, mild to moderate hyperandrogenism may manifest with hirsutism at puberty and is characterized by excessive hair growth in androgen-sensitive areas, but without additional signs of masculinization. Hirsutism may be idiopathic or the first sign of a polycystic ovary syndrome (1).

In 46,XX adolescents, virilization can result from severe hyperandrogenism because of underlying excessive production or abnormal metabolism of adrenal or gonadal androgens. Paradoxically, in 46,XY females, virilization around puberty points toward a possible condition of severe undervirilization during sex determination and differentiation in fetal life, manifesting at birth with a female

phenotype that may go unrecognized until puberty. At that time, high circulating androgens may then lead to the development of masculine bodily characteristics including deepening of the voice, clitoromegaly, severe acne, and severe, early-onset hirsutism.

Very rarely, drug abuse and environmental toxins may play a role and should be considered in unsolved cases (2). Timely investigation of the girl with virilization at puberty is essential, particularly if associated with a sudden onset of symptoms and/or a rapid progression because this should raise immediate concern of an androgen-secreting tumor of the ovaries or adrenal cortex (3-6).

Adrenal and Gonadal Tumors

Adrenocortical tumors are very rare (<0.2% of pediatric malignancies) and produce mostly not only excess androgens but also glucocorticoids (80%), thus manifesting with signs of hypercortisolism (Cushing syndrome) (6). Furthermore, they are often associated with particular genetic syndromes such as the Beckwith-Wiedemann syndrome or with a familial genetic predisposition to cancers (mostly from p53 mutations) (6).

Likewise, (androgen-secreting) tumors of the ovary are rare (4). The most common pediatric ovarian neoplasms are teratomas. These are germ cell tumors (GCTs) that originate from pluripotent germ cells. The majority are benign and hormonally inactive. Dysgerminoma is the most common malignant GCTs, developing from a preexisting gonadoblastoma, predominantly in persons with gonadal dysgenesis. In fact, a GCT may be the first manifestation of an undiagnosed disorder of sex development (DSD) and should raise suspicion and specific workup (7). Sertoli-Leydig cell tumors are exceedingly rare malignancies that produce androgens and cause severe virilization. They have a high association with

the DICER1 syndrome, a genetic disorder with increased risk for developing tumors in the lungs, kidneys, ovaries, thyroid, and several other locations (8).

Congenital Adrenal Hyperplasia

The most common genetic cause of virilization before and at puberty is congenital adrenal hyperplasia (CAH) (Table 1). In addition, rarer forms of DSD affecting gonadal development and/or sex steroid synthesis and action need to be considered. CAH is in most cases caused by 21-hydroxylase deficiency resulting from autosomal recessive variants of the *CYP21A2* gene, which has a prevalence of its classic form of 1 in 15,000 births worldwide (3, 9). Numerous genetic variants lead to glucocorticosteroid deficiency and adrenal androgen excess (3, 9). Virilization of the external genitalia at birth is therefore frequently seen in girls with more severe forms of classic *CYP21A2* deficiency, and diagnosis is usually made by clinical manifestation and neonatal screening in most countries (3, 9). By contrast, prevalence of the less severe, nonclassic/late-onset form of CAH is estimated more than 10-fold higher in the general population, and it is diagnosed later in childhood, in adolescence, or even only in adulthood (9). Nonclassic CAH typically manifests with hyperandrogenism resulting in premature pubarche, accelerated linear growth, advanced skeletal maturation, and signs of virilization including clitoromegaly (10).

Much rarer forms of CAH manifesting as late-onset virilization in individuals with a 46,XX karyotype include deficiencies of 3 β -hydroxysteroid dehydrogenase type 2 and 11 β -hydroxylase caused by variants in the *HSD3B2* and *CYP11B1* genes, respectively (3, 11, 12). Even variants in the cytochrome P450 oxidoreductase (*POR*) gene, a cofactor supporting several enzymes of steroidogenesis (including *CYP21A1*, *CYP17A1*, and *CYP19A1*) could explain virilization of affected females at puberty,

Table 1. Genetic disorders of sex development associated with pubertal virilization

Gonadal dysgenesis
-Structural or numerical aberrations of sex chromosomes
-Variants in genes involved in gonadal development (eg, <i>WT1</i> , <i>NR5A1</i> , <i>DMRT1</i>)
46,XX congenital adrenal hyperplasia (CAH)
-21-hydroxylase deficiency (<i>CYP21A2</i>)
-11-hydroxylase deficiency (<i>CYP11B1</i>)
-3 β -hydroxysteroid dehydrogenase deficiency (<i>HSD3B2</i>)
-Cytochrome P450 oxidoreductase deficiency (<i>POR</i>)
46,XX aromatase deficiency (<i>CYP19A1</i>)
46,XY defects of androgen synthesis and action
-17 β hydroxysteroid dehydrogenase deficiency (<i>HSD17B3</i>)
-5 α reductase deficiency (<i>SRD5A2</i>)
-Partial androgen receptor (<i>AR</i>) insensitivity syndrome (PAIS)

although so far only a polycystic ovary syndrome–like phenotype has been described in young women, whereas both 46,XY undervirilization and 46,XX virilization resulting from genetic variants of *POR* are typical at birth (3, 13). In addition, girls harboring autosomal recessive *CYP19A1* variants may also present at puberty with virilization because aromatase deficiency leads to elevated androgens and low estrogens (14). However, more typically these girls experience virilization in utero, have ambiguous genitalia at birth, and may suffer from ovarian cysts during childhood.

Disorders of Androgen Synthesis and Action, Testicular Dysgenesis, and Ovotesticular DSD

Sudden virilization at puberty can be seen in 46,XY girls who have a disorder of androgen synthesis or action (Table 1). To the former group belong genetic variants of the *SRD5A2* gene, catalyzing for DHT production from testosterone (15) and the *HSD17B3* gene, converting androstenedione into testosterone (16). Why testicular testosterone or DHT production is more efficient than prenatally in pubertal girls who have these conditions has been attributed in part to isoenzyme activation at puberty (3), but remains overall insufficiently understood. Massive virilization and clarification of the underlying diagnosis may lead to sex/gender reassignment in these individuals in adolescence in up to 50% of cases (17).

Partial androgen insensitivity syndrome is a form of testicular DSD caused by pathogenic mutations in the *Androgen Receptor* gene, resulting in a decreased sensitivity to the actions of androgens and has a prevalence of 1 to 5:100,000 (18, 19). The 46,XY females with partial androgen insensitivity syndrome and typical female-looking external genitalia at birth may present signs of external genital masculinization, including clitoromegaly or posterior labial fusion later in childhood or puberty. (17).

Various forms of gonadal dysgenesis (ie, incomplete testicular or ovarian differentiation) can also lead to virilization of girls with puberty after initial manifestation with a typical female phenotype at birth (Table 1). Chromosomal aberrations and pathogenic variants in several genes involved in the determination of the (bipotential) gonads and their differentiation may be the underlying cause (20). Some of these genetic anomalies will cause isolated DSD, whereas others are associated with additional developmental defects in other organ systems and may form a characteristic syndrome. For example, heterozygous dominant-negative mutations of the Wilms tumor suppressor gene (*WT1*) on chromosome 11 can lead to the Denys-Drash syndrome, which is associated with kidney and gonad malformations and a high risk for developing a Wilms tumor (21). The

46,XY babies with Denys-Drash syndrome have varying degrees of gonadal dysgenesis, leading to ambiguous external genitalia or even a female phenotype with variable virilization during later pubertal development depending on presence of functional testicular tissue (21). Moreover, spontaneous virilization at puberty has been reported in several cases with nonsyndromic 46,XY DSD resulting from *NR5A1/SF1* variants (22, 23). In these cases, testis histology revealed dysplastic gonads with Leydig cell hyperplasia at puberty. Interestingly, development of an ovotestis (ovotesticular DSD) has also been reported in a 46,XY subject with a deletion of *NR5A1* and in some 46,XX subjects harboring the *NR5A1* variants p.Arg92Trp/Gln (22).

Clinical Evaluation

A thorough history and a focused clinical examination are crucial for successful diagnostic evaluation of patients with signs of severe androgen excess (24). Fig. 1 summarizes investigations that are recommended in the workup of a girl with virilization at puberty. Main differential diagnoses are included in the overview.

Medical History

The medical history should include pregnancy and family history (eg, tumors, fertility issues), birth weight and gestational age at birth, somatic development and growth, age of adrenarche and thelarche, as well as menarche and menstrual characteristics (if already applicable). The timing and progression of the signs of virilization, such as clitoromegaly, acne, and hirsutism, along with a record of previous therapies (eg, hair removal procedures), are important for the diagnostic evaluation and management. History should include questions regarding virilization of the external genitalia (eg, clitoromegaly) or deepening of voice and breast atrophy.

Physical Examination

In the physical examination, blood pressure, height, weight and body mass index, waist-to-hip ratio, and signs of virilization, such as severe acne, hirsutism, and alopecia, should be assessed. The Ferriman and Gallwey score may be used to score hirsutism (25). It is also recommended to search for signs of Cushing syndrome such as rounded face, pink or purple stretch marks on the skin, muscle wasting, and centripetal fat distribution. Furthermore, it is important to determine the pubertal stage and perform a thorough examination of the external genital region looking for the presence of clitoromegaly and other signs of virilization or ambiguity.

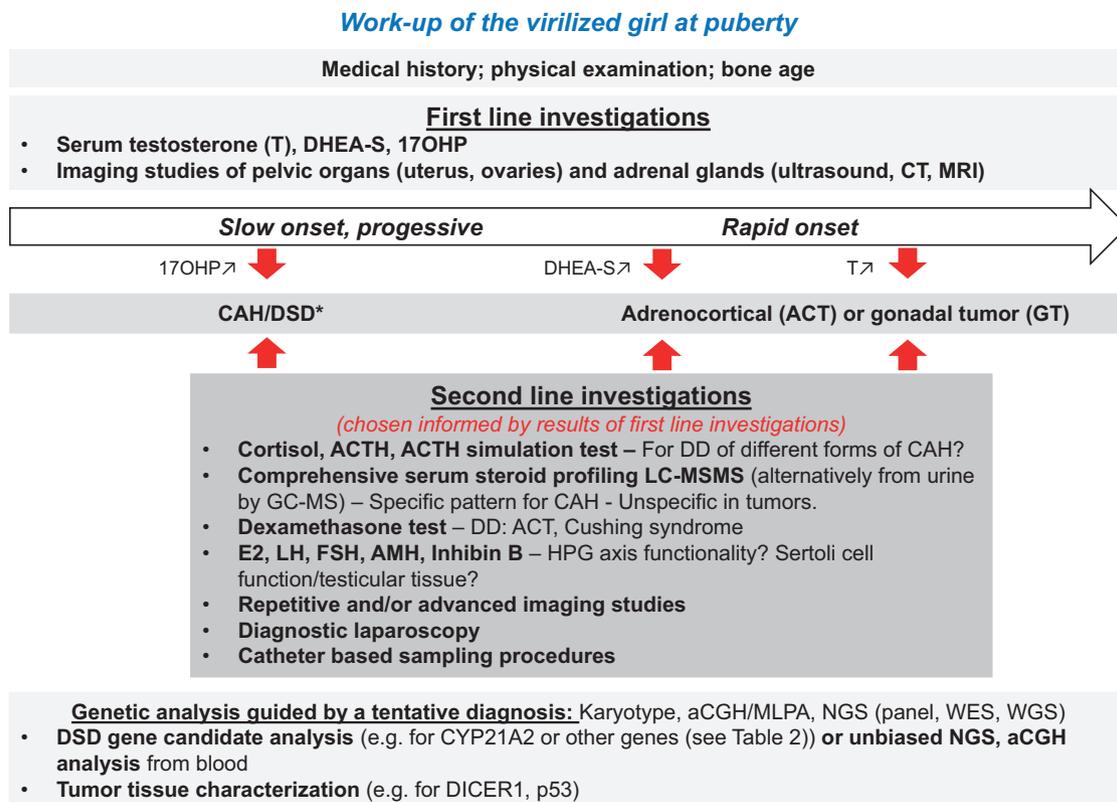


Figure 1. Flowchart for the workup of virilization of a girl at pubertal age.

Laboratory Investigations and Imaging Studies

Laboratory evaluations are essential in the workup of virilization (24). First-line investigations should include at least serum testosterone, dehydroepiandrosterone-sulfate (DHEA-S) and 17-hydroxyprogesterone (17OHP). Serum total testosterone levels are often more than 3 times elevated in patients with androgen-secreting ovarian tumors (26). DHEA-S (and adrenal androgen precursors such as DHEA and androstenedione together with glucocorticoids) are markedly elevated with adrenal tumors (27). Markedly increased 17OHP serum levels (3, 9) (or maybe 21-deoxycortisol in the future (28)), suggest CAH resulting from 21-hydroxylase deficiency. This diagnosis may be confirmed by a simple ACTH test and/or a targeted genetic test, if biochemical results are equivocal or genetical counseling is desired (9). Guided by the clinical findings and first laboratory results, imaging studies should ensue, including abdominal ultrasound, computed tomography and/or magnetic resonance imaging of the adrenal glands and the pelvic organs to exclude androgen-secreting ovarian or adrenal tumors.

Second-line investigations (29) may include an ACTH stimulation test for evaluating adrenal gland function in cases of mildly elevated or normal basal 17OHP to exclude milder forms of (nonclassic) CAH (9), or a more comprehensive plasma or urine steroid profile assessed by

a chromatographic mass spectrometric method to find specific patterns of rarer forms of CAH (Table 1) (30, 31). Patients suspected for Cushing syndrome should be evaluated with a 24-hour urine free cortisol, a diurnal ACTH and cortisol profile, and/or a low-dose dexamethasone suppression test (3). Baseline gonadotrophins (FSH, LH) and estradiol should be measured and interpreted according to age-specific reference intervals. Suppressed or unmeasurably low serum LH and FSH may be found before puberty onset or with severe androgen excess leading to a negative feedback blockade of the hypothalamic-pituitary-gonadal axis. Elevated LH and FSH is consistent with primary gonadal failure. Furthermore, measurement of serum Anti-Müllerian hormone (AMH) help in classifying the different forms of DSDs with and without gonadal dysgenesis (32). Normally, AMH is produced in high amounts in immature Sertoli cells, but physiologically declines once pubertal testosterone production starts. In 46,XY DSD, AMH is low in gonadal dysgenesis, but normal or elevated with defects of androgen synthesis or action. In chromosomal and 46,XY DSD with suspected gonadal dysgenesis, AMH levels indicate the existence of functional testicular tissue, (32, 33). Granulosa cells of primary and small antral ovarian follicles produce only small amounts of AMH from late fetal life until menopause. In 46,XX DSD elevated AMH levels indicate ovotesticular DSD with usually functional ovarian

tissue but dysgenetic testicular tissue (32). Tumor markers such as α -fetoprotein, β -human chorionic gonadotrophin, lactate dehydrogenase, inhibin, and cancer antigen 125 are positive in 50% to 80% of malignant ovarian lesions, but may also be positive in 20% of benign ovarian germ cell tumors (4). Repetitive imaging studies may be necessary, in case of unsolved tumor suspicion and/or localization. If still unsuccessful, catheter procedures for adrenal vein sampling may be considered for diagnosing and localizing suspected adrenal tumors, whereas diagnostic laparoscopy and biopsy may be performed for suspected gonadal tumors.

Genetic tests are now standard for diagnostic workup of many disorders to confirm a diagnosis at the molecular level and allow for genetic counseling and prognostic evaluation. This may include a simple karyotype, an array comparative genomic hybridization, a candidate gene or an unbiased next-generation sequencing (eg, whole exome or genome sequencing) approach. The appropriate method for genetic testing depends on the suspected diagnosis and is best advised by a geneticist.

Returning to the Patient

First-line investigations showed a very high serum testosterone and normal values for DHEA-S and 17OHP, orientating the diagnosis toward a gonadal rather than an adrenal origin of androgen excess (Table 2). Gonadal dysgenesis was suspected because of elevated LH and FSH (FSH > LH), and undetectable estradiol (E2). AMH was low (0.53 ng/mL; normal value <9.00 ng/mL). Ultrasound revealed a prepubertally sized uterus, normal adrenals, and gonads that were first described as normal, but on later review of images not clearly detectable; no tumor was found. Bone age was concordant with chronological age according to the Greulich-Pyle method. The 24-hour urine steroid profile excluded any form of nonclassic CAH and Cushing syndrome, but confirmed very high excretion of androgen metabolites (>3-fold of normal). The ACTH stimulation test showed normal reactivity of adrenal steroids, and the dexamethasone suppression test revealed normal inhibition of adrenal steroidogenesis. Overall, all these investigations pointed toward a gonadal origin of testosterone production, whereas elevated LH and FSH paradoxically suggested hypergonadotropic hypogonadism. Thus, gonadal dysgenesis owing to a DSD was suspected and genetic workup initiated.

Initially, conventional chromosomal analysis of 30 lymphocytes (180 mitosis) revealed a 45,X karyotype compatible with TS (Fig. 2A). Further genetic examinations in search of “hidden” Y-chromosome material included fluorescent in situ hybridization (FISH) and array

Table 2. Laboratory values before and after gonadectomy

	Reference	Initial (basal)	ACTH test basal/ stimulated	Dexametha- sone test	Postoperative		Postoperative day 75 before E2	Under E2 5 mo E2 6.25 μ g/24 h		Under E2 14 mo E2 12.5 μ g/24 h		Under E2 21 mo E2 25 μ g/24 h	
					day 1	<1.5 20*		<1.5 2.3
ACTH (ng/L)	7.2-63.6		2.1/...	1.5			2.3
Cortisol (nmol/L)	133-537 (6-10 AM) * 68-327 (4-8 PM)		227/668	26	167		167	...	206
DHEA (nmol/L)	3.9-20	3.8	4.0/6.8	3.2	3.3		3.3	3.8	4.5	4.5	4.5	2.87	2.87
DHEA-S (μ mol/L)	0.92-7.6	1.32	1.02/...	0.85	1.04		1.04	1.03	1.85	1.85	1.85	1.22	1.22
Androstenedione (nmol/L)	<8.4	<1.05	<1.05/1.12	<1.05	<1.05		<1.05	<1.05	<1.05	<1.05	<1.05	<1.05	<1.05
Free testosterone (pmol/L)	1.06-4.88	23.3	14.8/12.5	12.6	2.34		2.34	2.16	1.36	1.36	1.36	4.35	4.35
11-desoxycortisol (nmol/L)	<12	7.9	.../18.0	2.5	5.3		5.3	7.0	4.6	4.6	4.6	0.70	0.70
17-OH-progesterone (nmol/L)	<6	1.9	2.1/6.9	1.7	0.7		0.7	1.0	0.7	0.7	0.7	0.4	0.4
LH (U/L)		12.5	13.1/-	15.3	26		26	23.5	19.1	19.1	19.1	27.9	27.9
FSH (U/L)		37.5	39.4/-	42	79.6		79.6	72.4	67.4	67.4	67.4	70.8	70.8
Estradiol (pmol/L)		<20	<20	<20	<20		<20	<20	208	208	208	65	65

Numbers in bold mark values outside the reference range.
Abbreviations: DHEA-S, dehydroandrosterone sulfate; E2, estradiol.

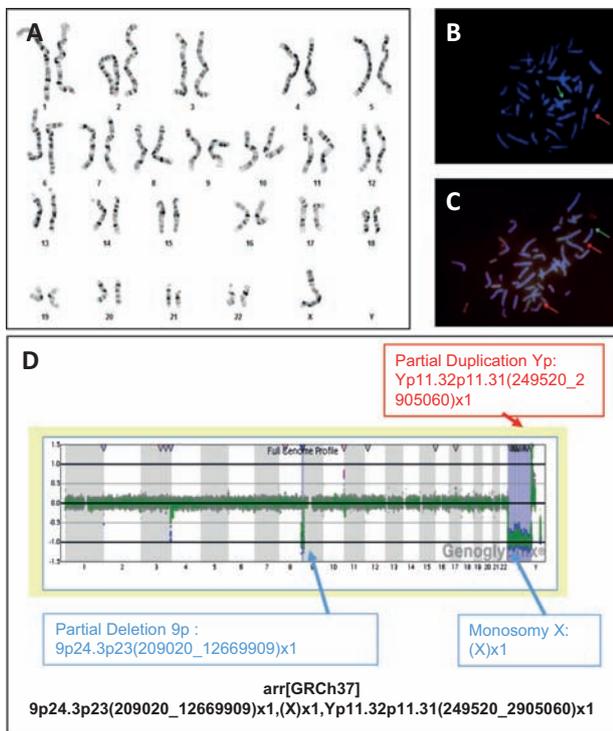


Figure 2. Genetic analyses performed in the girl presented in the case vignette. (A) Karyotype 45,X. (B-C) FISH analysis confirmed the presence of SRY and showed a suspicious hybridization pattern. (B) FISH analysis with LSI SRY(red)/CEPX(green) probes showing an unusual hybridization pattern. LSI SRY-signal on chromosome 9pter. (C) FISH analysis with SubTel-9p/9q-probe (9pter(green)/9qter(red)). No SubTel 9p signal present on the derivative chromosome 9, only a red signal, no green signal. (D) Array-CGH analysis. Full genome profile from Genoglyphix, CGX, 180K, PerkinElmer. Abbreviation: FISH, fluorescent in situ hybridization.

comparative genomic hybridization (180 K) analyses (Fig. 2B–D). They uncovered a terminal heterozygous deletion of 9p24.3p23 and the presence of Yp11.32p11.31, with the karyotype described according to the International System for Human Cytogenomic Nomenclature (2016) as follows: 45,X,ish der (9)t(Y;9)(305J7-T7,SRY+). arr[GRCh37]9p24.3p23(209020_12669909)x1,(X)x1,Yp11.32p11.31(249520_2905060)x1. Ectopic presence of *Sex Determining Region on Y* (SRY) has been shown sufficient to induce testis development (34). However, the terminal heterozygous deletion of 9p24.3p23 resulted in partial monosomy of 9p (~12.46 Mb) with absence of 49 genes including the pro-testis gene *Doublesex and Mab-3 Related Transcription Factor 1* (DMRT1), and explaining the female phenotype in our patient (Fig. 2).

Given that these results were consistent with a complex form of gonadal dysgenesis with functionally active testicular tissue, the patient underwent laparoscopy. Macroscopically atypical gonads were found on both sides (Fig. 3). Gonadectomy was performed to avoid further virilization and malignant degeneration because there is

a high risk for germ cell cancer (GCC), arising from surviving pluripotent germ cells in dysgenetic gonads harboring Y chromosomal material (35). Morphological and immunohistochemical analysis (Fig. 4) revealed a left gonad mostly differentiated as testis and characterized by Sertoli cell only tubules, extensive Leydig cell hyperplasia, and discrete signs of dysgenesis (intracapsular growth of tubules, ovarian-type stroma) at the gonadal periphery, as well as a small area of streak gonadal tissue. The right gonad was composed of streak tissue only with limited and scattered granulosa cells. Follicles or isolated germ cells were not detected by specialized immunohistochemical staining. There were no signs of in situ or invasive GCC, as indicated by negative SALL4 and OCT3/4 staining (not shown).

On follow-up, after removal of the gonads, testosterone values normalized (Table 2) and clitoromegaly reduced. The patient received psychosexual care and identified herself clearly in the female gender. Given the high LH/FSH values suggesting pubertal activation of the hypothalamic-pituitary-gonadal axis, E2 replacement therapy was started 2 months after gonadectomy to enable the development of secondary female sexual characteristics and to promote normal bone mass acquisition. The patient's compliance with transdermal E2 patch treatment was initially rather poor, but improved under psychological guidance, so that E2 and FSH levels normalized under stepwise dose adjustment (Table 2).

Discussion

We describe a girl who presented with spontaneous virilization at puberty. High serum testosterone, LH and FSH, and a complex chromosomal rearrangement, including presence of Y chromosomal material initially suggested a diagnosis of TS with mosaicism for a second cell line. A normal or partly deleted Y chromosome can be found in 6% to 11% of women with TS. However, the girl lacked clinical features associated with classic TS, such as typical facial characteristics, short stature, and cardiac or renal anomalies. Instead, the unique chromosomal rearrangement, resulting in the combined presence of the testis-inducing gene SRY with absence of the pro-testis gene DMRT1 on 9p had led to partial gonadal (testicular) dysgenesis, manifest as unilateral testis development with extensive Leydig cell hyperplasia resulting in high androgen production at puberty. The molecular genetic diagnosis and pathological findings are in line with hormonal results (ie, hypergonadotropic hypogonadism and testosterone levels well above the female reference range), but cannot explain why virilization did not occur already prenatally.

Early diagnosis and appropriate management of partial gonadal dysgenesis is crucial because of a strongly

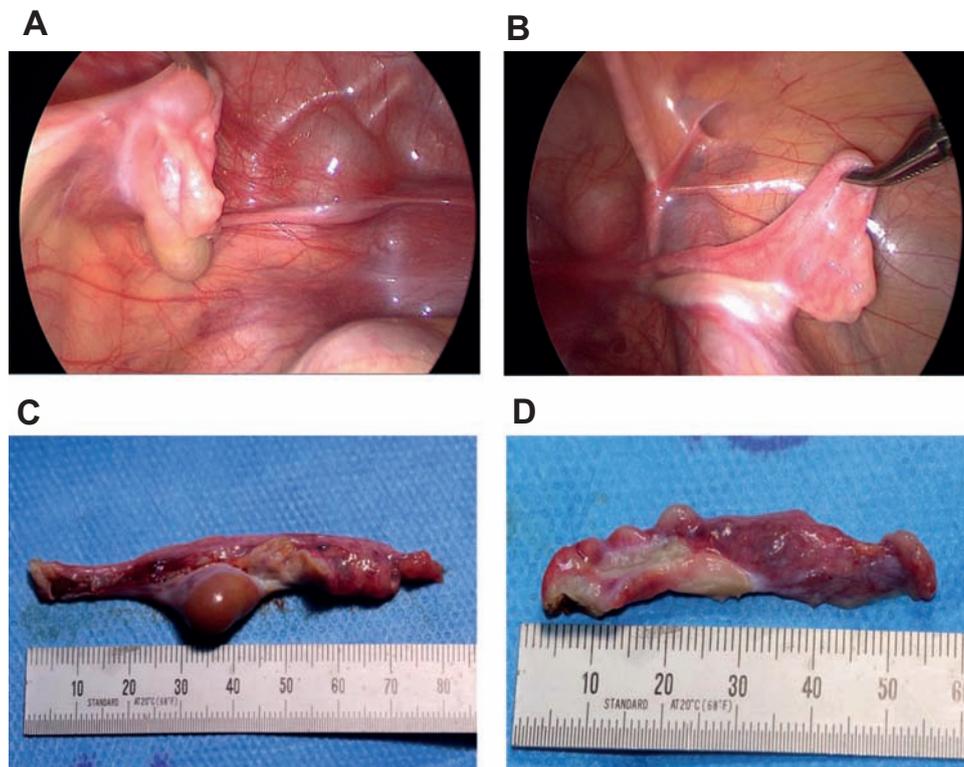


Figure 3. Pictures of gonads of the described patient at timepoint of laparoscopic gonadectomy. (A) Laparoscopic view on the in situ dysgenetic gonad on the left. (B) Laparoscopic view on the streak gonad on the right. (C) Left gonad after removal. (D) Right gonad after removal.

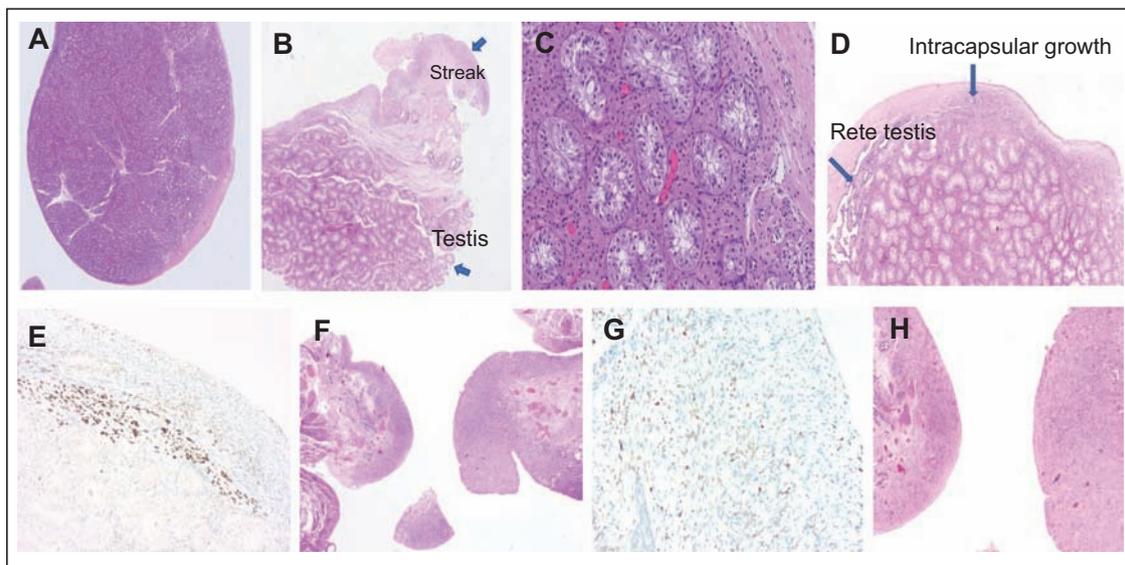


Figure 4. Histologic workup of the dysgenetic gonads. (A-E) Left gonad. (A-B) Left gonad predominantly developed as testis, with a smaller zone of streak tissue at the periphery. (C) Detailed analysis shows Sertoli cell-only tubules and diffuse intertubular Leydig cell hyperplasia. (D) The testicular periphery reveals rete testis and a more dysgenetic area with intracapsular growth of testis tubules in a background of ovarian-type stroma. (E) The latter finding is confirmed by the presence of scattered FOXL2 positive (brown) granulosa cells. (F-H) Right streak gonad. (F) Follicles or isolated germ cells are not detected. (G) The stromal background contains some dispersed FOXL2 positive (brown) granulosa cells confirming the overall female differentiation of this gonad. (H) Tube with fimbrial funnel on the right.

increased (up to 50%) risk of gonadoblastoma and invasive GCC development (36, 37) Therefore, a systematic search for hidden Y-chromosome material should be performed in

all TS girls with signs of virilization or with an unidentifiable marker chromosome identified by classical cytogenetic analysis (38-40).

Gonadal (testicular) dysgenesis with increased risk of gonadoblastoma can also be associated with partial or complete deletion of distal chromosome 9 (41). The recurrent distal 9p microdeletion syndrome has a prevalence of <1/1,000,000 and is characterized by dysmorphic features such as trigonocephaly, long philtrum, psychomotor retardation, speech problems, and atypical genitalia (42). The phenotype shows variable expressivity and is related to the size of the deletion, with cases of isolated 46,XY sex reversal without other dysmorphic features (43). Within this region, the strongest candidate for disrupted gonadal development is the *DMRT1* gene, mapping to 9p24.3 (44). *DMRT1* is a transcription factor expressed by both Sertoli and germ cells. It is critical for testis determination, differentiation, and maintenance. *DMRT1* haploinsufficiency is associated with abnormal testicular development, leading to germ cell loss and reduced or absent virilization of external genitalia (45-47). The particular genotype of sex reversal resulting from loss of *DMRT1* is extremely rare, with only a few cases reported. Marsudi et al (45) reported on a 12-year-old female with short stature and cognitive impairment, mimicking TS. Genetic analysis revealed loss of the *DMRT1* gene in a mosaic 45,XY,-9[8]/46,XY,r (9)[29]/47,XY,+idic r (9)×2[1]/46,XY,idic r (9)[1]/46,XY[1] karyotype. Further research is required to better understand the exact mechanism that underlies sex reversal caused by *DMRT1* haploinsufficiency (48), as well as the delayed onset of virilization in our case.

Conclusion

Virilization at puberty is more complex than routinely thought. All efforts should be taken to identify the underlying cause as a malignant tumor may be found and ongoing virilization may result in irreversible bodily changes. Underlying ovarian or adrenal tumors should be excluded by imaging studies. Karyotyping and a thorough search for Y chromosomal material by quantitative PCR or FISH are essential components of the genetic evaluation. If Y material is found, prophylactic gonadectomy of dysgenetic gonads may be advised to prevent gonadoblastoma and invasive tumor development. The described case report highlights the importance of repeated and expanded biochemical and genetic workup to solve unusual cases. Complex genetic rearrangements can cause unique, unexpected phenotypes.

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Additional Information

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