



Patients with disorders of sex development

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Disorders of sex development (DSDs) are a genetically and clinically heterogeneous group of congenital conditions of the urogenital tract and reproductive system. Time and spatially controlled transcription factors, signal molecules, and an array of different hormones are involved in the development of sex characteristics, and variations in their pathways and actions are associated with DSD. These conditions may be caused by numerical or structural variations in sex chromosomes as well as autosomes, variations in genes involved in gonadal and/or genital development, and changes in gonadal and/or adrenal steroidogenesis. Endogenous or exogenous (maternal) and possibly endocrine disruptors may also interfere with genital development.

Keywords: Sex development, Disorders, Gonads

Highlights

All individuals with a suspected disorders of sex development need to undergo a thorough diagnostic evaluation, including extensive whole-body and genital physical examinations, biochemical and genetic investigations, and imaging studies.

Introduction

Disorders of sex development (DSDs) are a genetically and clinically heterogeneous group of congenital conditions of the urogenital tract and reproductive system. Time and spatially controlled transcription factors, signal molecules, and an array of different hormones are involved in the development of sex characteristics, and variations in their pathways and actions are associated with DSDs. These conditions may be caused by numerical or structural variations in sex chromosomes as well as autosomes, variations in genes involved in gonadal and/or genital development, and changes in gonadal and/or adrenal steroidogenesis. Endogenous or exogenous (maternal) and possibly endocrine disruptors may also interfere with genital development.¹⁾

DSDs range from common disorders like cryptorchidism to very rare and complex conditions like complete XX or XY sex reversal. Cryptorchidism is estimated to exist with a prevalence of 0.7% to 3% in term boys and simple hypospadias affects one in 250 boys; however, overtly ambiguous genitalia may occur in one in 4,500 live births, and complete XX or XY sex reversal with unequivocal male or female phenotype at birth is estimated to exist in one in 20,000 live births.²⁾ These phenotypic variations are also difficult to estimate, as cases of the latter are often detected later in infancy, childhood, or even in adolescence due to unusual pubertal development and/or infertility.

Sex development

Sex development starts with the initial setting of either a 46,XX or a 46,XY karyotype. Sex development of the human embryo is divided into 3 stages. In the indifferent stage (lasting

Received: 2 November, 2020
Accepted: 23 November, 2020

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ISSN: 2287-1012(Print)
ISSN: 2287-1292(Online)

from fertilization to about 6 weeks of gestation), all embryos are morphologically indistinguishable.³⁾ In the next period, called sex determination (lasting from approximately 6 to 8 weeks of gestation), the bipotent gonadal anlagen eventually develops into ovarian or testicular cells. In the final stage, termed sex differentiation, the hormonal patterns in turn shape the individual phenotype, usually as an expression of male or female traits.⁴⁾

Classification of DSD

According to the Chicago classification (2006), DSDs can be classified into 3 categories: sex chromosome DSDs, which include Turner syndrome and Klinefelter syndrome, as well as 45,X/46,XY and 46,XX/46,XY variants. 46,XY and 46,XX DSDs can be further subdivided into the subclasses of disorders of gonadal development, disorders of androgen biosynthesis and excess, and unclassified. Further division is based on the use of clinically descriptive terms, including the molecular basis of the disorder where known.⁵⁾

Clinical presentation

Differences in sex development result from predominantly genetic pathogenic variants, but DSDs do not always manifest at birth and may be detected at different developmental stages of the life cycle.⁶⁾ The initial diagnosis and management of patients with ambiguous genitalia are challenging. In many cases, the genital ambiguity is obvious but the phenotype may be highly variable, ranging from predominantly male through truly ambiguous to predominantly female. However, it is very important to acknowledge that the same infant with congenital adrenal hyperplasia (CAH) may present as a phenotypic girl with clitoromegaly or phenotypic boy with apparently bilaterally undescended testes, which are only in due course found to be absent. Thus, the perception of the initial investigation can be highly subjective.⁷⁾

An apparently female newborn with clitoromegaly and posterior labial fusion or an inguinal hernia requires assessment for DSDs. An apparently male newborn should be investigated if (bilateral) cryptorchidism, hypospadias associated with a micropenis, or severe isolated micropenis is present.⁸⁾ The assessment of isolated minor hypospadias is controversial. Guidance from the United Kingdom Society for Endocrinology recommends that patients with isolated perineal hypospadias or any form of familial hypospadias need to be regarded as having a DSD.

The first examination of babies with DSDs after delivery is very important because questions will be asked of physicians by families regarding the baby's clinical status and sex but also because some conditions such as CAH may be potentially life-threatening.⁹⁾ Clinical situations can frequently be difficult to manage, especially in those cases where the sex of rearing is uncertain. An assessment should be performed as promptly as possible and should include both clinical evaluation as well as

psychological support of the families. Optimal care for patients with a suspected DSD is best provided by an experienced clinical team with adequate knowledge about the range of conditions associated with DSDs.¹⁰⁾ The team may exist as a clinical network with links between more than 1 specialist center. As a minimum standard, the clinical team should include pediatric and adult specialists in endocrinology, surgery and/or urology, clinical psychology/psychiatry, genetics, radiology, nursing, and neonatology.

Diagnostic approach

The aims of the diagnostic evaluation are as follows:

- To rule out any life-threatening conditions, such as adrenal insufficiency or electrolyte imbalance
- To establish the diagnosis in order to decide further management, including possibilities of gender development
- To provide psychological support and counseling to the family

History

Newborns with CAH can present with decreased feeding, vomiting, and failure to gain weight. The mother's antenatal history should be reviewed thoroughly for any exposure to drugs such as progesterone or antiandrogens in the first trimester of pregnancy that can cause undervirilization of a male fetus. A maternal history of virilization in pregnancy can be a feature of placental aromatase deficiency or the presence of an adrenal tumor in the mother, which can cause virilization in a female fetus.¹⁰⁾ Since a majority of DSDs are inherited, the family history is important as it gives a clue of the inheritance pattern and diagnosis. A history of early sibling deaths with or without atypical genitalia points to CAH. Similarly, a history of parental consanguinity and DSDs in the siblings indicates an autosomal recessive cause like simple virilizing CAH or 5 α -reductase deficiency, whereas infertility in maternal aunts points to an X-linked disorder like androgen-insensitivity syndrome (AIS).⁷⁾

General physical examination

The examination should begin with measurement of the vital signs, including the heart rate, capillary refill time, and blood pressure, to rule out adrenal insufficiency due to CAH. On the other hand, hypertension may be a manifestation of CAH due to 11 β -hydroxylase deficiency or 17 α -hydroxylase deficiency. The pediatrician should always conduct a head-to-toe examination before proceeding to a genital examination. This serves 2 purposes: firstly, this will convey to the parents that the overall health of the baby, not just their atypical genitalia, is of importance. Secondly, a detailed examination helps to identify associated congenital anomalies and dysmorphism syndromes. Smith-Lemli-Opitz syndrome, due to a block in cholesterol synthesis, is associated with abnormal facies (e.g., microcephaly, broad nasal bridge, low set ears, cleft palate,

and small chin), syndactyly, heart defects, growth retardation, hypotonia, and developmental delays. Mutations in the *SOX9* gene are associated with undervirilization in 46,XY children and skeletal malformations known as campomelic dysplasia (bowing of long bones). Skeletal deformities such as craniosynostosis, femoral bowing, radiohumeral synostosis, and choanal atresia are also seen in infants with Antley-Bixler syndrome due to a mutation in the *POR* gene. Microcephaly, facial dysmorphism, short stature, and mental retardation are associated with gonadal dysgenesis in patients with 9p deletions. Congenital heart defects are commonly seen in 46,XY individuals with *GATA4* or *FOG2* mutations and those with 45,X/46,XY mixed gonadal dysgenesis. Midline defects such as cleft lip and palate or a single middle incisor (in an older child) can be seen in those with hypogonadotropic hypogonadism due to hypopituitarism. Furthermore, many syndromic forms of DSDs have not been elucidated to date at the molecular level.

Genital examination

The genital morphology can vary from predominantly female with a palpable gonad to predominantly male with absent gonads. The pediatrician needs to perform a detailed genital examination and document the findings with the help of figures in the case sheet. The genital evaluation includes the measurement of the phallic size; identification of the presence, location, and size of palpable gonads as well as the number and site of urogenital openings; and an assessment of the labioscrotal folds.¹¹⁾ Any asymmetry of the gonads would point to a diagnosis of mixed gonadal dysgenesis (45,X/46,XY). The stretched penile length (SPL) is measured by pressing a hard ruler against the pubic symphysis and gently stretching the phallus against the scale. The SPL should be compared to age-related normative data and considered as a micropenis if below -2.5 standard deviation. At birth, an SPL of less than 2.5 cm in term boys is considered an example of a micropenis. In a phenotypic girl, a clitoris measuring greater than 1 cm is considered enlarged. The clitoris may appear larger because of a small amount of adipose tissue in the labia in preterm babies. It should also be kept in mind that increased dehydroepiandrosterone sulphate (DHEA-S) levels may lead to clitoromegaly, and clitoral size frequently decreases in the first postnatal month with decreasing DHEA-S levels in preterm babies.¹¹⁾ Clitoromegaly in extremely preterm babies should be evaluated carefully because there are insufficient data on normal clitoris size in extremely-low-birth-weight and very-low-birth-weight babies.

Presence of gonads

A palpable gonad always has a testicular component and, if present, provides the most important etiological clue of a DSD. The palpation of gonads must be done carefully by first gently occluding the superficial inguinal ring of one side so as to prevent the testes from retracting and then palpating on the

same side.

Number and position of urogenital openings

The presence of one or more openings and the exact location of the urethral meatus (distal penile, midshaft, or perineal) need to be documented. A single opening in an apparent female indicates a persistent urogenital sinus.

Labioscrotal folds and anogenital ratio

The labioscrotal folds should be inspected for rugosity, pigmentation, and labial fusion. Hyperpigmented genitalia points to cortisol deficiency due to CAH and result from melanocyte-stimulating hormone excess, which is cosecreted with adrenocorticotrophic hormone (ACTH) due to a lack of feedback inhibition. Posterior labial fusion indicates exposure to androgens in the first trimester of pregnancy. The anogenital ratio is defined as the distance between the anus and posterior fourchette (where the labia meet) in females (in males, this would be the junction of the perineum and rugated skin of the scrotum) divided by the distance from the anus to the base of the clitoris/phallus. An anogenital ratio of greater than 0.5 indicates virilization of the genitalia.¹²⁾

Two scoring systems are useful to indicate the degree and severity of atypical genitalia, the Prader score and the external masculinizing score (EMS).³⁾ The Prader score was first used to classify 46,XX DSDs due to CAH and consists of stage 1 (predominantly female with mild clitoromegaly) to stage 5 (predominantly male without gonads).¹³⁾ The EMS has been used to classify the severity of male undervirilization based on the size of the phallus, position of the urethral meatus, presence of the gonads, and degree of scrotal fusion. Each of these carries a score of up to 3 points for a total possible score of 12 points, with lower scores indicating a more severe form of undervirilization; typically, an EMS score of less than 10 points would indicate the need for further evaluation. These would include infants with a micropenis with or without undescended testes, any form of hypospadias with undescended testes, or severe perineoscrotal hypospadias but not isolated forms of mild hypospadias. An isolated micropenis can be a manifestation of hypogonadotropic hypogonadism but occasionally of 5 α -reductase deficiency or partial AIS (PAIS) as well.

Laboratory investigations

In all infants with ambiguous genitalia and/or bilateral impalpable gonads, the first approach should be to conduct karyotype and follicle-stimulating hormone (FISH) analyses for sex chromosome assessment. Furthermore, a pelvic ultrasound can rapidly evaluate the internal genital status. If a uterus is seen in a (partially virilized) newborn, the most likely diagnosis is CAH and potentially life-threatening electrolyte imbalances should be checked for.¹⁴⁾ This first round of investigations should also include assessments of plasma glucose, serum

17OH-progesterone (17OHP), and serum electrolytes. Serum 17OHP is usually unreliable before the age of 36 hours as well as in very premature infants and, in the salt-losing form of CAH, serum electrolytes usually do not become abnormal before day 4 of life.¹⁵⁾ In many countries, neonatal screening based on the determination of 17OHP has been called for. In situations where the level of suspicion of CAH is very high and the infant needs immediate steroid replacement therapy, further serum samples should be collected and stored before beginning such therapy. These should be of a sufficient volume to assess, in order of priority, 17OHP; testosterone; androstenedione; and, possibly, the renin activity or concentration. If rarer forms of CAH are suspected, ACTH and cortisol as well as other steroid precursors may be determined. A urine steroid profile can be helpful in the differential diagnosis of all forms of CAH, but this diagnostic test is only available in a few settings.

When 2 X chromosomes are present but no or only rudimentary Müllerian structures are identified and the hormonal evaluation shows high concentrations of androgens in an apparently female patient, ovotesticular or testicular DSDs may be suspected. The genital phenotype may be highly variable. If FISH and quantitative fluorescent polymerase chain reaction indicate the presence of SRY, the diagnosis of an XX ovotesticular or testicular SRY-positive DSD can be made. If the result is an SRY-negative DSD, further molecular approaches may be required. A gonadal biopsy is recommended to verify the diagnosis.

In infants with sex chromosomes other than 46,XX, a different array of investigations is necessary to determine the presence of testes and the adequacy of hormone production and action. Luteinising hormone (LH), follicle-stimulating hormone (FSH), anti-Müllerian hormone (AMH) or inhibin B, as well as the androgens androstenedione, testosterone, and dihydrotestosterone, could be measured in first-line studies to evaluate testicular function. However, especially the determination of steroid hormones can be difficult depending upon the laboratory method used. Nowadays, the gold standard for steroid measurement in serum or plasma is liquid chromatography in combination with mass spectrometry, which may not be available in developing countries and at every institution. Therefore, each determination should be discussed with the laboratory regarding its availability and validity.

The concentration of gonadotrophins (LH and FSH) is low in both sexes at birth, increase around 1 week of age, and peak between 1 week and 3 months. The period of activity in the hypothalamic-pituitary-gonadal axis occurring between 1 week and 6 months of life, referred to as mini-puberty, is an opportunity for gonadal assessment. Consequently, during the period of mini-puberty, hormone levels may be determined without stimulation testing; however, later, a human chorionic gonadotropin test is mandatory for sex steroid assessment during infancy. Interestingly, in almost all patients with complete AIS (CAIS), the expected rise in the postnatal concentrations of testosterone and LH does not occur and assessment might require stimulation testing. Alternatively, genetic profiling employing next-generation sequencing may be performed as a

first-line diagnostic approach in cases with 46,XY DSD.

Imaging studies

Ultrasound is a useful and safe imaging modality to examine the internal structures, though highly observer-dependent. It is important to document the presence of gonads, Müllerian structures, and adrenal size. If the Müllerian structures are absent, it indicates that AMH was produced in fetal life from functioning testes, whereas, presence of a uterus indicates that the testes are either absent or dysgenetic.¹⁶⁾

Magnetic resonance imaging (MRI) of the pelvis is indicated to localize the gonads in case the testes are not appreciated on ultrasound or if there is a suspicion of gonadal dysgenesis or malignancy. Müllerian structures are also better visualized on MRI. MRI is especially useful in the older child or adolescent, as, in younger children, anesthesia may be required and the quality of assessment may not be superior to ultrasound.

In girls with CAH and persistent urogenital sinus, a genitogram or micturating cystourethrogram is useful to outline the confluence of the vagina to the urethra in order to plan surgical correction. Newer methods such as perineal ultrasound may be used to visualize the confluence in a similar pattern.

Genetic testing

Once the sex chromosome constitution is known, hormonal evaluation and, where available, targeted sequencing of DSD genes would be optimal to guide decision-making towards a diagnosis. Nowadays, the usual Sanger sequencing of single genes is more and more dismissed in favor of a targeted approach of investigating multiple genes in a single investigation. In several publications, panels incorporating multiple genes have been described or, alternatively, exome sequencing has been proposed. This holds true mostly for 46,XY DSDs, where the underlying molecular pathophysiology is hard to distinguish and the phenotypic overlap is broad. In classical CAH due to 21-hydroxylase deficiency, single-gene analysis is useful. The evaluation of genetic testing employing next-generation sequencing approaches requires both bioinformatics expertise as well as linkage to clinical knowledge. Currently, in about 30% of 46,XY DSD cases, a significant mutation in a DSD gene can be identified by employing modern sequencing techniques.

DSDs presenting later in life

DSDs may be diagnosed for the first time in childhood, e.g., in a girl who presents with inguinal hernia and is found to have testes or in a boy with bilateral cryptorchidism who is found to have Müllerian structures on ultrasonography. In India and other developing countries, it is not uncommon for even children with significant genital ambiguity to present late, which may have significant implications on the gender of rearing and the psychosocial health of the affected individuals.

About 6% to 8% of all cases of DSD come to light for the first time in adolescence due to atypical, delayed, or absent pubertal development.

In girls, the most common presentation is as primary amenorrhea, with or without breast development and with or without virilization.¹⁷⁾ Evaluation should be initiated at 13 years of age if there is no pubertal development and at 16 years if breast development has progressed normally but there is amenorrhea. In girls with one or more signs of virilization, such as clitoromegaly, excessive acne, facial hair, and voice change along with a lack of secondary sexual development, evaluation may be considered before the age of 13 years.¹⁸⁾ The common causes of primary amenorrhea (other than DSDs), including constitutional delay, systemic illness, anorexia, celiac disease, androgen-excess polycystic ovarian syndrome, hypogonadotropic hypogonadism, and primary ovarian insufficiency, should always be considered during evaluation.¹⁹⁾

History-taking should include a family history of DSDs, parental consanguinity, and a history of having noted any genital ambiguity in infancy or having undergone any surgical procedures. Also, examinations should include assessments of height, body mass index, blood pressure, sexual maturity rating (of breasts as well as pubic hair), and external genitalia to look for any ambiguity/virilization (e.g., clitoromegaly, posterior labial fusion, labial or inguinal swellings).²⁰⁾

The investigative work-up should include LH, FSH, estradiol, and testosterone and ultrasound of the pelvis should be performed by an experienced sonologist. Karyotyping should be done if either the gonadotropins are raised or the uterus is absent.²¹⁾ Knowledge of the clinical findings in conjunction with these investigations help in making a provisional diagnosis. The most common DSD to present in concert with primary amenorrhea is the sex chromosome DSD, Turner syndrome. The most common 46,XY DSDs that present in adolescent girls are CAIS and complete gonadal dysgenesis (CGD); CAIS is characterized by normal breast development, absent or diminished sexual hair development, and absent uterus, while CGD presents with a lack of breast development, hypergonadotropic hypogonadism, and usually with a rudimentary uterus.²²⁾ The most common 46,XY DSD that presents with virilization and a lack of thelarche in adolescent girls are 2 disorders of androgen biosynthesis, 5 α -reductase type 2 deficiency and 17 β -hydroxysteroid dehydrogenase type 3 deficiency.²³⁾ Many of the patients afflicted with these 2 disorders do not have any noticeable ambiguity at birth but experience significant virilization at the time of puberty.²⁴⁾ In a few other 46,XY DSDs, including PAIS, partial gonadal dysgenesis, alterations in steroidogenic factor-1 (SF-1/NR5A1), and ovotesticular DSDs, the ambiguity at birth may be milder and hence overlooked but becomes more prominent at adolescence. In all these conditions, Müllerian structures will usually not be detectable. The differential diagnoses of virilization at puberty include nonclassical CAH and androgen-secreting tumors of the ovaries or adrenal glands. However, the uterus will be normal in these conditions.

Children with 46,XX simple virilizing CAH raised as boys

may first present in adolescence with the complaint of a small phallus, bilateral cryptorchidism, and poor height gain. In untreated CAH, as the testosterone levels are high, breast development is generally not seen even though the ovarian function is normal. Bone age is, however, markedly advanced and most children experience precocious development of pubic hair and early growth spurt but have a short final height.

Physiological gynecomastia and concern about a small phallus are relatively common in pubertal boys, especially in those who are overweight or obese. However, when a boy with gynecomastia is noted to have a small phallus, this should raise a red flag for the possibility of 46,XY DSD, especially if there is hypospadias and/or undescended or small testes or a past history of hypospadias repair or orchidopexy. PAIS may be first diagnosed in adolescence due to a lack of pubertal progression and gynecomastia. Boys with Klinefelter syndrome (47,XXY and variants) may also present in adolescence with a tall stature, pubertal gynecomastia, small phallus, and small testes. The investigative work-up will be guided by the differential diagnosis.

Conclusion

It is recommended that DSD cases should be treated at specialized centers by an experienced multidisciplinary team prepared to deal not only with the diagnostic investigation but also all subsequent aspects of therapy. All individuals with a suspected DSD need to undergo a thorough diagnostic evaluation, including extensive whole-body and genital physical examinations, biochemical and genetic investigations, and imaging studies, with the results discussed by the multidisciplinary team.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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