HHS Public Access

Author manuscript

Best Pract Res Clin Obstet Gynaecol. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Best Pract Res Clin Obstet Gynaecol. 2018 April ; 48: 90–102. doi:10.1016/j.bpobgyn.2017.11.005.

DISORDERS OF SEX DEVELOPMENT

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Abstract

Normal sex development depends on the precise spatio-temporal sequence and coordination of mutually antagonistic activating and repressing factors. These factors regulate the commitment of the unipotential gonad into the binary pathways governing normal sex development. Typically, the presence of the SRY gene on the Y chromosome triggers the cascade of molecular events leading to male sex development. Disorders of sex development comprise a heterogenous group of congenital conditions associated with atypical development of internal and external genitalia. These disorders are generally attributed to deviations from the typical progression of sex development. Disorders of sex development can be classified into several categories including chromosomal, gonadal, and anatomic abnormalities. Genetic tools such as microarray analyses and next-generation sequencing techniques have identified novel genetic variants among patients with DSD. Most importantly, patient management needs to be individualized especially for decisions related to sex of rearing, surgical interventions, hormone treatment, and potential for fertility preservation.

Keywords

Disorders of sex development; Ambiguous genitalia; congenital adrenal hyperplasia; urogenital anomalies

INTRODUCTION

Disorders of sexual development (DSD) encompass a group of congenital conditions associated with atypical development of internal and external genital structures. These conditions can be associated with variations in genes, developmental programming, and hormones. Affected individuals may be recognized at birth due to ambiguity of the external genitalia. Others may present later with postnatal virilization, delayed/absent puberty, or infertility. The estimated frequency of genital ambiguity is reported to be in the range of

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CONFLICT OF INTEREST: NONE

1:2000-1:4500 [1]. According to the Danish Cytogenetic Central Registry, the prevalence of XY females is 6.4 per 100,000 live born females. In this registry, the prevalence of androgen insensitivity was 4.1 per 100,000 live born with median age at diagnosis of 7.5 years. The prevalence of XY gonadal dysgenesis was 1.5 per 100,000 live born females with median age at diagnosis of 17 years [2]. The incidence of DSD varies among ethnic groups with the highest incidence in the southern African population.

International stakeholders representing multiple disciplines continue to modify the terminology used to categorize specific DSDs to emphasize the underlying genetic etiologies [3]. Ongoing development and use of novel molecular cytogenetic techniques have enriched understanding regarding the genomic alterations associated with DSDs. In addition, analyses regarding these genomic alterations have illuminated novel genetic regulatory mechanisms associated with DSDs [4].

When presented with a child with ambiguous genitalia, unique decision-making challenges can occur regarding sex of rearing, parent and patient education, and medical management [5]. It is important to note that sex does not indicate gender; sex refers to the biology of the internal and external genital structures that is traditionally considered to be a binary categorization. Gender identity is the self-defined experience of one's gender. Tales from Greco-Roman cultures, e.g. Hermaphrodite and Daphne, have documented and celebrated transformations and fluidity in sex and gender identity [5].

EMBRYOLOGY

Sexually dimorphic development of the reproductive tracts is influenced by multiple factors. Normal sex development is dependent on the synergistic orchestration of activating and repressing factors interacting in a precise spatio-temporal pattern [6]. Sex determination is governed by the sex chromosomes. The Sex Determining Region on the Y chromosome (SRY) gene located on the short arm of the Y chromosome is the binary switch that initiates the male developmental program [7]. The pivotal experiments performed by Dr. Alfred Jost established the relevance of testosterone for male sexual differentiation [8].

The urogenital ridges develop by 4-6 weeks of gestation as outgrowths of the coelomic epithelium. Subsequently, the urogenital ridges develop into the kidneys, adrenal cortices, gonads, and reproductive tracts. SRY functions as a transcription factor to trigger the developmental trajectory that directs differentiation of the bipotential gonad into a testis during the 6^{th} week of human gestation. SRY induces SOX9 expression; SOX9 activates and maintains the male gonadal differentiation pathway. With differentiation of the Sertoli cells, the developing testis becomes organized into two compartments. One compartment consists of the testis cords that are aggregates of the germ cells surrounded by Sertoli cells and encased by the peritubular myoid cells. The other compartment is the testis interstitium, which contains the Leydig cells and testis vasculature.

Initially, both Wolffian and Müllerian ducts develop. The Wolffian ducts originate as the excretory ducts of the mesonephros. Testosterone, secreted by the fetal Leydig cells, stabilizes the Wolffian ducts resulting in the development of the epididymis, vas deferens,

ejaculatory duct, and seminal vesicle. Another hormone secreted by the testis, insulin-like factor 3 (INSL3), mediates testicular descent from the original perinephric location through the abdomen. Testosterone promotes testicular descent into the scrotum. Testicular descent is generally completed by 32 weeks' gestation. Sertoli cells secrete Anti-Müllerian Hormone (AMH), which induces regression of the Müllerian ducts.

Ovarian differentiation occurs slightly later than testicular differentiation. In the absence of SRY in the female fetus, the ovary specific transcription factors, Forkhead transcription factor 2 (FOXL2), Wingless type MMTV integration site family, member 4 (WNT4), R-spondin 1 (RSPO1), and the activated β-catenin pathway, initiate and maintain ovarian differentiation [9]. In the absence of testosterone and dihydrotestosterone (DHT), the external genital structures develop into the clitoris, vagina, and labia. Both the urethra and the vagina open onto the perineum.

In peripheral target tissues, testosterone is converted to DHT. DHT promotes fusion of the urethral folds to form the corpus spongiosum and penile urethra. DHT also promotes development of the genital tubercle into the corpora cavernosa of the penis and fusion of the labioscrotal folds to form the scrotum.

Primordial germ cells migrate from the allantois to the fetal gonads. Differentiation of germ cells to a spermatogenic or an oogenic fate does not depend on their XY or XX karyotype. Rather, the neighboring somatic cells in the gonads influence germ cell differentiation. In the female embryo, germ cells are exposed to high levels of retinoic acid which induce the expression of STRA8 leading to germ cell meiosis and development of oocytes. In the developing testis, the absence of retinoic acid causes the germ cells to develop into gonocytes that differentiate into spermatogonia and proliferate by mitosis; meiosis only starts at puberty in the male gonad.

GENETICS OF SEX DIFFERENTIATION AND DEVELOPMENT

Sex development is achieved by the precise synergistic temporal-spatial expression of numerous activating and repressing factors. Deviations from this established developmental sequence can result in disorders of sex development. Investigations into the molecular basis of DSDs in patients have elucidated many genes and genetic regulatory mechanisms involved in this process. Gene expression reflects tissue specificity, programing, and relative dosages to influence cell fate decisions.

Genes involved in the initial differentiation of the bipotential gonad include empty spiracles homeobox2 (EMX2), chromobox homolog2 (CBX2), Wilms' tumor 1 (WT1), steroidogenic factor 1(NR5A1), LIM homeobox factor 9 (LHX9), sine oculis-related homeobox 1/4 (SIX 1/4), and GATA binding protein 4 (GATA4) [10]. Subsequently, cell fate decisions influence the differentiation of the bipotential genital ridge towards male or female phenotype. This process involves a complex regulatory network in which activation of one pathway, i.e., testicular, leads to repression of the other pathway, ovarian, and vice versa [11].

In the developing testis, SRY promotes SOX9 expression. In conjunction with SRY and NR5A1, SOX9 generates a positive feedback loop for maintaining its expression and to

promote Sertoli cell development. Two paracrine signaling molecules downstream of SOX9, fibroblast growth factor 9 (FGF9) and prostaglandin D2 synthase (PGD2) promote maintenance of testicular development [12]. FGF9 signals from the central region of the gonad to promote SOX9 expression and antagonize WNT4 signaling. Other genes relevant for testicular differentiation include CITED4 and other members of the *SOX* family, i.e. SOX3, SOX10, and SOX13 [13].

Rather than being the "default pathway", differentiation of the ovary is an active process dependent on the activity of specific factors. WNT4 suppresses SOX9 expression in the pre-granulosa cell of the developing ovary. WNT4 and RSPO1 stabilize β-catenin expression. FOXL2 is another ovarian transcription factor and nuclear protein crucial for differentiation and maintenance of ovarian differentiation [14]. The proteins, WNT4 and RSPO1, promote β-catenin accumulation in the nucleus where it interacts with LEF1 to promote transcription of other genes. FOXL2 and β-catenin also repress SOX9 expression. The WNT4 pathway upregulates follistatin, which inhibits activin B and prevents formation of the testis-specific vasculature [15]. Recent data derived from mouse studies suggest that the orphan nuclear receptor, chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) may play an active role in eliminating the Wolffian ducts in females [16].

CLASSIFICATION OF DSD

DSDs are classified into several categories (Table 1). The category of 46,XX DSD includes virilized females such as girls with a virilizing congenital adrenal hyperplasia and girls with aberrant ovarian development. The category of 46, XY DSD patients includes patients with abnormal testicular differentiation, defects in testosterone biosynthesis, and impaired testosterone action. Sex chromosome DSDs include Turner Syndrome, Klinefelter Syndrome, and 45,X/46,XY gonadal dysgenesis. In general, patients with Turner Syndrome and Klinefelter Syndrome do not present with genital ambiguity. Other DSDs include XX sex reversal, XY sex reversal, and ovotesticular disorder.

A. XX, DSD

The most common form of virilizing congenital adrenal hyperplasia is 21-hydroxylase deficiency due to mutations in the 21-hydroxylase (CYP21A2) gene. Infant girls with classic salt-losing 21-hydroxylase deficiency usually present in the immediate neonatal period due to genital ambiguity. For affected female infants, virilization of the external genitalia ranges from clitoromegaly to perineal hypospadias with chordee to complete fusion of labiourethral and labioscrotal folds. The magnitude of external genital virilization may be so extensive that affected female infants appear to be males with bilateral undescended testes [17]. Unless identified by neonatal screening, infant boys with congenital adrenal hyperplasia typically present at 2 to 3 weeks of age with failure to thrive, poor feeding, lethargy, dehydration, hypotension, hyponatremia, hyperkalemia, and normal male sexual development. Hyperpigmentation of the scrotum may be apparent. When the diagnosis is delayed or missed, congenital adrenal hyperplasia is potentially fatal. Newborn screening programs decrease the morbidity and mortality associated with acute adrenal insufficiency or with assignment of affected female infants to male sex of rearing [18].

Other disorders of steroidogenesis associated with 46,XX DSD include 11-beta hydroxylase deficiency, 3-beta hydroxysteroid dehydrogenase deficiency, P450 oxidoreductase deficiency, and aromatase deficiency due to mutations in CYP11B1, HSD3B2, POR, and CYP19A1, respectively. Paradoxically, male infants with HSD3B2 and POR mutations may present with undervirilization due to impaired testosterone biosynthesis [19]. Maternal hyperandrogenism during gestation can be due to luteomas of pregnancy, androgen secreting tumors, and exposure to exogenous androgen.

Loss of function mutations in the genes coding for ovarian factors are associated with ovarian dysgenesis and/or accelerated loss of primordial follicles resulting in premature ovarian failure. After birth, WNT4 is detected in oocytes and granulosa cells [20]. SERKAL syndrome is characterized by female to male sex reversal associated with renal, adrenal, and lung dysgenesis; this disorder is associated with a homozygous recessive missense mutation in WNT4 [21]. Mutations in FOXL2 are associated with blepharophimosis-ptosis-epicanthis inversus syndrome that can be associated with premature ovarian failure (BPES I). Continued FOXL2 expression in the ovary is essential to maintain an ovarian phenotype because loss of FOXL2 expression in adult mice reprograms granulosa and theca cells into cells that are similar to Sertoli and Leydig cells, respectively [22]. Other genes associated with ovarian dysgenesis and premature ovarian failure include LHX8, MCM8, MCM9, NOBOX, and FSHR [23,24,25].

B. XY, DSD

This category includes patients with abnormal testicular differentiation, defects in testosterone biosynthesis, and impaired testosterone action. The phenotype may be limited to aberrant testicular differentiation or may include other anomalies. Loss of function SOX9 mutations are typically associated with gonadal dysgenesis and campomelic dysplasia. Mutations in GATA4 may also be associated with congenital heart disease in addition to testicular anomalies. Patients with Smith-Lemli-Opitz associated with 7-dehydrocholesterol reductase (DHCR7) mutations typically manifest characteristic facial features, and syndactyly of the second and third toes. Several phenotypes have been described for patients with WT1 mutations including Denys-Drash, Frasier, Meacham, and WAGR syndromes. Other genes associated with XY gonadal dysgenesis include CBX2, DHH, DMRT1, DMRT2, MAP3K1, and SOX8 [26]. In XY individuals, MAP3K1 mutations appear to shift signaling pathways to suppress SOX9 and promote ovarian differentiation [27].

Mutations in the proteins necessary for testosterone biosynthesis are associated with undervirilization. The genes encoding these proteins include SF1 (NR5A1), LH receptor (LHR), steroidogenic acute regulatory peptide (StAR), cholesterol desmolase (CYP11A1), 17α-hydroxylase/17,20-lyase (CYP17A1), 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2), 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3), 3α-hydroxysteroid dehydrogenase (AKR1C2/4), P450-oxidoreductase (POR), and 5α-reductase type 2(SRD5A2) [28,29,30].

Mutations in the androgen receptor gene (AR/NR3C4), which is located on the long arm of the X chromosome at Xq12, interfere with testosterone signaling [31]. The phenotype of patients with AR/NR3C4 mutations ranges from normal female external genitalia, labial

masses, and absence of a uterus to partial forms that may present with male infertility in adulthood.

C. Sex chromosome DSD

Turner syndrome describes the phenotype of patients with aneuploidy or structural rearrangements of the X chromosomes. Structural rearrangements include isochromosome Xq, partial deletions, and ring X chromosome. The reported incidence is 1 in 2500 live-born female birth [32]. Patients with Turner syndrome may be diagnosed in the neonatal period due to low birth weight, short neck, and lymphedema of hands and feet. Other typical presentations include short stature and delayed puberty. Characteristic features include epicanthal folds, downslanting palpebral fissures, low set ears, micrognathia, left-sided cardiac anomalies, and horseshoe kidneys. The cardiac anomalies include coarctation of the aorta, bicuspid aortic valve, and aortic stenosis. Girls suspected of having Turner syndrome should have a standard 20-cell karyotype. If mosaicism for Turner syndrome is suspected, FISH can be performed, additional metaphases can be counted, or a second tissue can be analyzed. Genetic analysis that detects Y chromosomal material warrants further evaluation because these girls may develop virilization and have an increased risk for gonadoblastoma and dysgerminoma. Girls with Turner syndrome have an increased risk to develop other disorders such as Hashimoto's thyroiditis, celiac disease, neurosensory hearing loss, hypertension, and diabetes mellitus. Approximately 50% of girls have congenital heart disease including coarctation of the aorta, bicuspid aortic valve, and increased risk for aortic dissection. The likelihood for development of these co-morbidities is greatest in the girls with 45,X karyotype [33]. Growth hormone therapy improves final height. Estrogen and progesterone hormone therapy are essential to promote development of secondary sexual characteristics and prevent osteopenia [34]. A large series of British women with Turner Syndrome reported a decreased incidence of breast cancer, but an increased risk for gonadoblastoma, corpus uteri cancer, and possibly childhood brain cancers [35].

Klinefelter syndrome is characterized by 47,XXY karyotype. The incidence is approximately 1 in 500 males. Affected boys have normal external genital development. They may present with tall stature, small testes, delayed puberty, infertility, and gynecomastia. Boys with Klinefelter syndrome often manifest dyslexia, behavior difficulties, and defects in executive function [36]. Autism spectrum disorders are more common among boys with Klinefelter syndrome than the general population [37].

D. XX, Sex Reversal, Ovotesticular Disorder, and XY, Sex Reversal Disorder

Patients with XX sex reversal can be classified into two major groups. One group (SRY+) is positive for SRY due to the translocation of the SRY gene to another location, which is usually the X chromosome or, rarely, an autosome. The other group consists of SRY negative XX (SRY−) males.

Ovotesticular disorder is defined by the presence of both ovarian follicles and seminiferous tubules in the same patient. The specific phenotype depends on relative gene expression patterns and the function of the gonads particularly related to hormone secretion. Gonadal histology can include ovarian, testicular, ovotesticular, and dysgenetic patterns. Potential

mechanisms responsible for ovotesticular disorder in the XX (SRY−) individual could involve activation of testis specifying genes in the absence of SRY and/or inadequate expression of pro-ovary/anti-testis genes. Duplications involving the SOX9 locus or potential SOX9 regulatory elements have been associated with XX testicular and XX ovotesticular DSD [13]. Genes associated with ovotesticular DSD include NR5A1, SOX3, SOX10, WNT4, and RSPO1 [38]. Considerations regarding sex assignment in the XX patient with ovotesticular disorder are similar to other patients with DSD with the caveat that sufficient ovarian tissue with follicles may be present to allow for pregnancy.

Duplication of a region on the Xp21.2 region of the X chromosome containing the DAX1 gene is associated with XY sex reversal. Deletion of this region in an XY individual is associated with congenital adrenal hypoplasia. However, a microdeletion of this region was associated with XX sex reversal underlining the genetic complexity of this locus [39].

Persistent Mὺllerian Duct Syndrome (PMDS)—PMDS is a rare autosomal recessive disorder characterized by the persistence of Mὺllerian structures in a boy. Typically, phallic development and testicular function are normal. This disorder is typically diagnosed during surgery for inguinal hernia and/or cryptorchidism. Often, both testes are on the same side (transverse testicular ectopia) and may be embedded in the broad ligament. Abnormal development of male excretory ducts is common. Although most men are infertile, fertility may be possible if at least one testis is scrotal with intact excretory ducts. Most cases are due to either AMH or AMHR2 mutations [40].

Urogenital anomalies—Some patients initially appear similar to patients with DSDs, but have disorders of urogenital tract development. Examples of malformations include cloacal and bladder exstrophy. The prevalence of uterine malformations has been reported to range from 5.5-9.8% [41]. Uterine developmental anomalies can range from complete aplasia, fusion defects, and septal absorption defects [42]. Associated findings can include renal, spinal, and cardiac anomalies. Uterine anomalies can be associated with MODY 5 diabetes, renal cysts, and HNF1B mutations. Mutations in the HOX genes have been associated with uterine anomalies. The hand-foot-genital syndrome associated with HOXA13 mutations is characterized by limb malformations and urogenital anomalies in both males and females [43]. In addition to HOX genes, other genes associated with uterine anomalies include WNT genes, GATA3, FRAS1, FREM, and other genes associated with syndromic ciliopathies [44].

GENETIC TESTING IN DSD

Genetic testing plays an important role in the evaluation of a patient with a possible DSD because knowing the genetic etiology improves the ability to predict the patient’s phenotype, clarifies recurrence risk, and can be utilized in medical decision-making.

Peripheral blood karyotype analyses can be useful to detect the X and Y chromosomes, balanced chromosomal rearrangements, and large structural rearrangements. Fluorescence in situ hybridization (FISH) analysis using X and Y centromere-specific probes can be used to assess for sex chromosome mosaicism. Probes specific for the SRY gene can be used to

ascertain for Yp rearrangements. Unknown marker chromosomes and chromosomal rearrangements should be identified using FISH analyses to discern Y chromosomal material and establish recurrence risk.

Chromosomal microarray analyses such as array CGH or SNP microarrays can detect submicroscopic gene variations. CGH may identify novel candidate genes associated with DSD. The use of customized CGH focused on DSDs can interrogate multiple genes simultaneously which can accelerate the diagnostic process and limit the financial burden associated with testing multiple individual genes [45]. One caveat is that CGH may fail to detect balanced chromosomal translocations and low level mosaicism.

Genome-wide association studies (GWAS) offer a hypothesis free approach for the detection of novel loci associated with DSD. Many loci detected in GWAS are located outside of the coding region of a gene which confounds the interpretation regarding the functional impact of the variant on the phenotype. These variants may influence gene regulatory elements, affect co-factor recruitment, or modulate local chromatin structure. The need to replicate GWAS findings limits its usefulness in DSDs because of phenotypic and genetic heterogeneity and the low incidences for specific disorders.

The use of next generation sequencing (NGS) techniques such as whole exome sequencing (WES) simultaneously targets the coding regions of thousands of genes. Whole genome sequencing (WGS) targets the entire genome. These techniques are useful, but neither technique adequately detects large copy number variants, repetitive sequences, e.g. trinucleotide repeats, aneuploidy, or epigenetic changes.

Variations such as translocations, inversions, duplications, and deletions can modify the normal chromatin structure and alter spatiotemporal relationships with gene regulatory elements [46]. Non-coding genomic changes near SOX9 have been associated with several phenotypes including XY male to female DSD associated with campomelic dwarfism and XX female to male sex reversal [47]. Genomic rearrangements involving long-range regulatory elements may result in ectopic and/or disrupted spatiotemporal expression of relevant genes. Two examples of AR mutations located outside of the coding region and associated with AIS include a mutation located in the 5′-untranslated region associated with reduced protein levels and an intronic mutation that created a novel 5′-splice site resulting in aberrant splicing and decreased AR protein levels [48,49]. A synonymous AR mutation, p.S510S, changed the exon 1 donor splice site generating a premature stop codon and truncated protein [50]. In some instances, no AR mutations have been detected suggesting that other proteins located beyond AR influence testosterone signaling [51].

ETHICS OF GENETIC TESTING

WES and WGS are powerful tools that are being used to identify the molecular basis of many diseases. However, these tests can detect genetic variants unrelated to sexual differentiation. Hence, counseling and informed consent need to precede these genetic studies; parents and patients should be alerted and counseled about the possibility of incidental findings with the potential for significant medical impact. Additional existing

limitations of NGS include the inability to completely characterize the functional significance of all variants and false assignment of causality detected by these tools [52].

GONADAL TUMORS

The decision to pursue gonadectomy needs to be individualized with active participation of a multidisciplinary team and, if possible, knowledge of the specific molecular etiology [53]. Considerations include risk of malignant degeneration, fertility potential, and ability to bring the gonads into a location for repeated physical examinations. The presence of a Y chromosome in a dysgenetic gonad is associated with high risk for neoplastic transformation into gonadoblastoma or dysgerminoma. Germ cell neoplasia in situ (GCNIS) in the testis represents a pre-malignant change. The neoplastic cells in these lesions are derived from primordial germ cells that arrested at an early stage of development and typically express OCT3/4.

Removal of testes in patients with complete androgen insensitivity is controversial. Most recent data indicate that the risk for tumor development is low until early adult years [54,55]. For patients with CAIS, the risks of neoplastic changes, risks of the surgical procedure, and need for long term hormone replacement should be discussed. Hence, many women with CAIS elect to keep gonads *in situ*. Laparoscopic gonadopexy to situate the gonads in a fixed position near the anterior abdominal wall with gonadal biopsy, molecular screening with SNP and micro-RNA testing, and ultrasound surveillance may be an option for patients wishing to avoid gonadectomy [56,57]. Delayed surgery promotes shared decision making with the patient, family, and healthcare providers [58].

SURGERY IN DSD

No uniform consensus regarding the indications, timing, and extent of the operation is applicable for individuals with DSD. Each patient warrants individual contemplation and attention by a multidisciplinary team at experienced centers. Considerations include future fertility, risk for gonadal tumors, propensity for urinary tract infections, avoiding stigmatization related to atypical genital anatomy, and ensuring functional genital anatomy to allow future penetrative intercourse [59]. For girls with CAH, favorable outcome was reported with early genital surgery [60,61]. Available outcome reports are largely small clinical series with diagnostic heterogeneity. Importantly, data regarding outcome for non-treated DSD are even more limited.

FERTILITY POTENTIAL AND PRESERVATION

Fertility preservation via embryo, oocyte, or sperm banking has become an option for those who face a loss of fertility due to a chronic illness such as cancer. Experimental protocols are being developed for preservation of fertility for prepubertal children with cancer. Apart from individuals with virilizing congenital adrenal hyperplasias, most individuals with DSDs have traditionally been considered to be infertile. Finlayson and colleagues propose a paradigm shift to this traditional notion because they detected germ cells in the gonads of patients with CAIS, ovotesticular disorder, Denys-Drash syndrome, and other disorders

associated with DSD [62]. Limitations of their report include small sample size and incomplete knowledge regarding the functional competence of these germ cells. Currently, most protocols for cryopreservation of immature gonadal tissues are experimental. Ethical considerations include false hope, potential transmission of genetic disorders to future offspring, financial burden, and provision of consent/assent for a minor child [63]. Nevertheless, optimism regarding potential assisted reproductive technologies may be justified following a report of a live birth following a uterine allograft transplantation in a female with uterine agenesis and successful intracytoplasmic sperm injection (ICSI) procedure with a subsequent live birth after testicular sperm extraction from a 46,XX/46,XY azoospermic male [64,65]. The multidisciplinary team involved in the care of children with DSDs should discuss fertility potential and possible role of assisted reproductive techniques [66].

APPROACH TO THE PATIENT

The birth of a child with a DSD is generally not a medical emergency. Rather, the birth of a baby with ambiguous genitalia is bewildering, alarming, and is considered to be a social emergency. Everyone wants to know if the baby is a boy or a girl. In many instances, the parents have been told the sex of the infant based on prenatal ultrasound findings. Until the sex of rearing has been established, the infant should be referred to as “your baby”.

Following review of the pregnancy and family histories and a thorough physical examination, it is appropriate to congratulate the parents on the birth of their child, show them the physical findings on their baby, and review that the gonadal structures are bipotential. Most importantly, “guessing” whether the child is a boy or a girl is inappropriate. A multidisciplinary team comprised of pediatric endocrinologists, pediatric urologists/surgeons/ob-gyn specialists, geneticists, pediatric radiologists, neonatologists, pediatric nurse educators, and behavioral health professionals should be involved in the care of infants, children, and adolescents with DSDs [67]. It is important to include the parents in discussions regarding sex of rearing and be cognizant of their level of understanding and cultural/religious perspectives and traditions [68,69].

The infant needs to be carefully examined for evidence of other anomalies [70]. The symmetry of the external genitalia, presence of palpable gonads, genital pigmentation, and extent of labioscrotal fusion should be assessed. The length and diameter of the phallus should be measured. The location of the urethral meatus and number of perineal openings need to be noted. The presence of posterior labial fusion and estimation of the ano-genital ratio can be helpful. For normal girls, the distance from the base of the phallus to the posterior forchette should be approximately 2/3 of the distance from the base of the phallus to the anus [71].

Individualized laboratory evaluations provide optimal information to ascertain the likely etiology and select the initial management recommendations. Bloodwork may include electrolytes, 17-hydroxyprogesterone, testosterone, LH, FSH, AMH, plasma renin activity, androstenedione, and DHT. Karyotype analysis and additional genetic testing are essential. Determination of urinary steroids and/or stimulation tests may be use to clarify the specific

diagnosis [72]. Ultrasound studies to assess for the presence of a uterus can be helpful. Ovaries are often too small to be accurately visualized on ultrasound; the lack of visualization of ovaries does not indicate absence of ovaries. Renal anomalies can be assessed by renal ultrasound.

The infant's history and physical examination can direct the course of the laboratory investigations. Significant virilization of the external genitalia with bilateral non-palpable gonads offers a clue that the infant is a virilized female with a virilizing form of congenital adrenal hyperplasia. Apparently normal symmetric female external genitalia with labial masses suggest the possibility of complete androgen insensitivity syndrome. Asymmetric external genital development can be associated with gonadal dysgenesis and ovotesticular disorder.

Upon completion of all studies, this information can be shared with the parents. The tone of this encounter should be positive and optimistic to promote bonding between the parents and their infant. During this discussion, sex of rearing, medical management plans, gonadal development, results of genetic testing, recurrence risks, and follow-up plans can be discussed. As the child transitions to an adolescent and young adult, specific details regarding their medical condition, karyotype, and potential for fertility need to be openly discussed.

For the older child or adolescent patient, an evaluation for delayed puberty, primary amenorrhea, or virilization can lead to the diagnosis of a DSD. A thorough evaluation including linear growth patterns, presence and sequence of secondary sexual characteristics, and family history is essential. On physical examination, the external genital appearance, pubertal features typical of estrogen effect such as breast development, and characteristics of androgen effect such as pubic hair and virilization should be noted. In this instance, the patient and parents play active roles in the diagnostic evaluation and decision-making process. Confidentiality and privacy are essential because the diagnosis of a DSD can be devastating to the patient and family. Discussion regarding karyotype and likely infertility are essential aspects of medical care for these patients.

SUMMARY

Disorders of sex development are variations in reproductive tract development. Novel genetic techniques have introduced a new era of the diagnosis of DSDs and elucidation of the molecular factors involved in sex development. Thoughtful respectful care is critical for the management of infants, children, adolescents, and their families to ensure positive and meaningful quality of life. Goals for individuals with DSDs include psychosocial well-being, sexual satisfaction, and fertility options [73].

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PRACTICE POINTS

- Normal sexual development is dependent on the synergistic orchestration of numerous activating and repressing factors interacting in a precise spatio-temporal pattern.
- Disorders of sex development can be classified into several categories and are associated with atypical development of chromosomal, gonadal, or anatomic sex.
- Patients with DSDs can present in infancy with ambiguous genitalia or at older chronological ages with aberrant pubertal development.
- The healthcare team needs to provide patients with comprehensive medical information regarding their specific condition as appropriate for age, developmental stage and cognitive abilities. Patients and their parents benefit from review of this information as they pass from childhood to adolescence to adulthood.
- Karyotype analyses, microarray analyses, and next generation sequencing techniques are helpful in the diagnostic and genetic evaluation of patients; these techniques may identify novel genes involved in sex development.
- The benefits and potential risks of surgical interventions need to be carefully reviewed and assessed with parents and patients.
- Multidisciplinary individualized health care is essential for all patients with DSDs. Goals include fostering the individual's healthy sexual and gender identity development while minimizing risks for deleterious physical and psychosocial consequences.
- Shared decision making and open communication are vital for optimal health and quality of life. This approach needs to respect the wishes, beliefs, and cultural traditions of patients and their families.

RESEARCH AGENDA

- Current evidence-based data remain inadequate to address assignment of male or female sex for some infants.
- Elucidation of the factors and processes involved in gender identity development is needed because our current knowledge of the structures and functions of the CNS underlying gender identity is extremely limited.
- Evidence-based data to address issues related to timing, techniques and consent for surgical interventions.
- Exploration of fertility preservation

Highlights

- Normal sex development depends on the precise spatio-temporal sequence and coordination of mutually antagonistic activating and repressing factors.
- Disorders of sex development can be classified into several categories including chromosomal, gonadal, and anatomic abnormalities.
- Genetic tools such as microarray analyses and next-generation sequencing techniques have identified novel genetic variants among patients with DSD.
- Patient management needs to be individualized especially for decisions related to sex of rearing, surgical interventions, hormone treatment, and potential for fertility preservation.

Table 1

Classification of Disorders of Sex Development Associated with Ambiguous Genitalia

XX, DSD
Androgen-induced
Virilizing Congenital Adrenal Hyperplasias
Placental Aromatase Deficiency
Glucocorticoid Receptor Mutation
Maternal androgen secreting Tumor
Virilizing luteoma of Pregnancy
Androgen Exposure (Norethindrone, Ethisterone, Norethynodrel,
Medroxyprogesterone, Danazol)
Ovotesticular Disorders
XY DSD
Impaired Testosterone Synthesis
Leydig cell agenesis
LH/HCG receptor (LHCGR) mutations
Congenital lipoid adrenal hyperplasia (StAR)
Cholesterol side chain cleavage mutations (CYP11A1)
3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) mutations
17α-hydroxylase/17,20 lyase (CYP17A1) mutations
P450 oxidoreductase (POR) mutations
Smith-Lemli-Opitz (DHCR7) mutations
17β-hydroxysteroid dehydrogenase type 3 (HSD17B3) mutations
5α-reductase type 2 (SRD5A2) mutations
Cytochrome b5 (CYB5A)
3α- hydroxysteroid dehydrogenase deficiency (AKR1C2 and AKR1C3)
Denys-Drash syndrome (WT1)
Sex Chromosome DSD
Turner Syndrome
Klinefelter Syndrome
Mosaicism, e.g. 45,X/46,XY
Triple XXX Syndrome
XXYY Syndrome
XX or XY Disorder of Gonadal Development
Complete gonadal dysgenesis
Partial gonadal dysgenesis Gonadal regression
Ovotesticular DSD
XY Persistent Mullerian Duct Syndrome
Low AMH (AMH)
Normal or High AMH (AMHR2)
Malformation Syndrome
CHARGE syndrome

Hand-foot-genital syndrome
MRKH Syndrome
MURCS Association
McKusick-Kaufman Syndrome
Aphallia
Cloacal/Bladder Exstrophy
Isolated Hypospadias
Penoscrotal Transposition