AMBIGUOUS GENITALIA IN THE NEWBORN

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ABSTRACT

Ambiguous genitalia constitute a rare phenotypic presentation of the urogenital system that can signal an underlying life-threatening disorder. Thus, it is imperative to determine the etiology as quickly as possible when ambiguity is noted. The formation of typical male or female external genitalia results from a number of genetic and physiological events starting with sex determination and progressing through differentiation of internal and external reproductive structures after a zygote is formed. While the inability to proceed through sex determination and differentiation in the usual manner is referred to as a disorder of sex development (DSD), not all people with DSD have ambiguous genitalia. The focus of this chapter is genital ambiguity associated with DSD in individuals who possess either a 46,XY or 46,XX chromosomal complement; however, DSD including genital ambiguity can be observed in people with other combinations of sex chromosomes such as 45,X/46,XY.

INTRODUCTION

Ambiguous genitalia constitute a rare phenotypic presentation of the urogenital system that can signal an underlying life-threatening disorder. Thus, it is imperative to determine the etiology as quickly as possible when ambiguity is noted. The formation of typical male or female external genitalia results from a number of genetic and physiological events starting with sex determination and progressing through differentiation of internal and external reproductive structures after a zygote is formed. While the inability to proceed through sex determination and differentiation in the usual manner (for example complete androgen insensitivity syndrome) is referred to as a disorder of sex development (DSD), not all people with DSD have ambiguous genitalia. The focus of this chapter is genital ambiguity associated with DSD in individuals who possess either a 46, XY or 46, XX chromosomal complement; however, DSD including genital ambiguity can be observed in people with other combinations of sex chromosomes such as 45,X/46,XY.

To appreciate the various genital phenotypes associated with ambiguity, it is important to first define typical genitalia for males and females, respectively (Table 1). A full-term male infant is expected to possess bilateral testicles that are descended, complete formation of scrotal folds including midline fusion, and a typical size penis (average penile length is 3.5 ± 0.4 cm, for a full term infant born in the U.S. (1), including well-formed corporal bodies and a urethral meatus located at the tip. An infant with bilateral cryptorchidism, bifid scrotum and hypospadias, or isolated penoscrotal hypospadias, should be investigated for DSD. Isolated micropenis, if both testes are descended and normal in size is not considered a presentation of

ambiguous genitalia. Similarly, distal hypospadias with no other atypical genital features in males is not usually indicative of DSD (2). A full-term female infant is expected to have bilateral separation of the labial folds, no palpable gonads, and separate urethral and vaginal openings. The average clitoral length and width for a full-term infant girl born in U.S. is 4.0 ± 1.24 mm and 3.32 ± 0.78 mm, respectively (1). Labial fusion or palpable gonads in what appears to otherwise be typical female external genitalia should be investigated further. Perceived clitoromegaly is not usually associated with an underlying DSD if the newborn girl was born prematurely (3).

It is important that a newborn with ambiguous genitalia be evaluated in a timely manner by a multi-disciplinary team (typically comprised of endocrinologist, psychologist, and urologist), preferably one with experience in DSD evaluation and treatment. The reason for this is two-fold: 1. to assign an appropriate sex of rearing to the infant based on the etiology of the condition and associated medical and psychosexual outcomes, and 2. to detect any underlying life-threatening disorder if present. Gender assignment should take into consideration predictions for long-term satisfaction with sex of rearing, sexual function, and fertility potential (1, 4, 5). It is generally agreed that professionals who have experience with DSD medicine (pediatric endocrinologists, pediatric urologists, psychiatrists, psychologists, geneticists) explain the steps of sex determination and differentiation to parents of affected newborns followed by an explanation, when possible, of the underlying cause of DSD for their child (6). It is important for these professionals to explain what is known about the long-term medical, surgical, and psychosexual experience of individuals affected by DSD, and to acknowledge when such information is unavailable (1). Finally, several websites exist for patients and their families that allow for the exchange of information between peers, provide coping strategies, and discuss decision-making processes that may improve outcomes for newborns with ambiguous genitalia. Such information can be found on websites supported by the Accord Alliance (www.accordalliance.org), DSD families (www.dsdfamilies.org), AIS DSD Support Group (www.aisdsd.org), and CARES Foundation (www.caresfoundation.org).

Table 1. Phenotypic Characteristic of Normal Full-Term Male or Female Genitalia and Ambiguous Genitalia

	Appearance of Genitalia
Full Term Male	Bilateral testicles that are descended Complete formation of scrotal folds with midline fusion Average penile length of 3.5 ± 0.4 cm
Full Term Female	Bilateral separation of labial folds No palpable gonads Separate urethral and vaginal openings

Newborn with Ambiguous Genitalia	Bilateral cryptorchidism in a male Bifid scrotum with hypospadias in a male Penoscrotal hypospadias in a male Labial fusion in a female Palpable gonads in a female

TYPICAL SEX DETERMINATION AND DIFFERENTIATION

Table 2: Major Steps Involved in Development of Internal and External Genitalia	
Sex Determination	Formation of a 46,XY or 46,XX zygote
Sex Differentiation: Gonadal Differentiation	Formation of either testes or ovary
Sex Differentiation: Ductal Differentiation	Maintenance of Wolffian structures /regression of Mullerian structures or vice versa
Sex Differentiation: Differentiation of External Genitalia	Masculinization of external genitalia under the influence of dihydrotestosterone in a male

Sex Determination

Sex determination refers to the commitment of an embryo to subsequent male or female development from a bipotential beginning. In humans, this is accomplished by the establishment of genetic sex. When fertilization occurs between an oocyte (carrying an X chromosome) and a sperm (carrying either an X or a Y chromosome) the result is a zygote that is usually genetically female (46, XX) or male (46, XY). The formation of bipotential gonadal ridges, Müllerian and Wolffian duct structures common to both 46, XX and 46, XY embryos are evident by week 5 of gestation (7). Multiple transcription factors are involved in the formation of these structures including LIM-1, EMX-2, PAX-2, WT-1, SF-1 and DAX-1 as well as the growth factors DMRT1, ATRX and insulin receptors (7-12). Gonadal primordium is dependent on expression of GATA binding protein 4 (GATA4), Wilms tumor 1 (WT1) and the nuclear receptor steroidogenic factor 1 (SF1, encoded by *Nr5a1*) for proliferation and survival (10, 13, 14). Insulin and IGF-1 signaling is also essential as loss of this signaling has been described to cause agenesis of adrenal glands and testes to ovary reversal (15).

Sex Differentiation

Sex differentiation, or the development of sex differences, is the next step in the formation of males and females. The process of sex differentiation involves gonadal differentiation, ductal differentiation, and differentiation of the external genitalia.

DIFFERENTIATION OF GONADAL SEX

Gonadal primordium is the somatic cell component of testis or ovary and is established by gestational week 4 in humans. The supporting cells which are the first to differentiate (sertoli cells in testes and granulosa cells in ovary) are responsible for the differentiation of the steroidogenic cell lineage cells and supports gametogenesis(16). Differentiation of ovaries or testes from the bipotential gonadal ridge tissue in humans is initiated by 6 weeks, and fully achieved by 13 to 14 weeks of fetal development. Testicular differentiation is controlled by a number of time and

dosage-sensitive genes (8). For example, testicular sex determination is initiated by *SRY* expression in somatic cells of the testes which then stimulates genes critical for testes development and represses genes important for ovarian development (7). *SRY* expression leads to *SOX9 expression*, resulting in upregulation of *FGF9*. These signals act together in a positive feedback loop to ultimately inhibit *WNT4* expression.

In the absence of *SRY* in people with a 46, XX chromosomal complement, *RSPO1* and *WNT4* are expressed to stabilize cytoplasmic β-catenin. Both *WNT4* and β-catenin suppress the *SOX9*/*FGF9* positive feedback loop, allowing ovarian differentiation to proceed. Most evidence indicates that ovarian differentiation occurs independently in several lineages including supporting cells and germ cells (17). A list of genes involved in sex reversal and the proteins they encode for are provided in Table 3.

DUCTAL DIFFERENTIATION

Fetal production of testicular androgens and anti-müllerian hormone (also known as Müllerian inhibiting substance) is required for the next steps of sex differentiation to occur. During the early stages of fetal development, both males and females possess Wolffian (the antecedent to male internal genital structures) and Müllerian (the antecedent to female internal genital structures) ducts. In males, androgens are necessary to support the development and maintenance of the internal Wolffian ducts, while anti-müllerian hormone (AMH) suppresses female (müllerian) duct development. The biosynthesis of testosterone by the Leydig cells requires the activity of a series of enzymes (18). Expression of androgenic effects by target tissues such as Wolffian duct structures requires the binding of testosterone to functioning androgen receptors (19).

In females, ovaries do not produce AMH during fetal development (18); hence, development and maintenance of the Müllerian duct structures are permitted. Furthermore, because the fetal ovaries do not secrete significant amounts of androgens, the Wolffian ducts become atrophic.

DIFFERENTIATION OF EXTERNAL GENITALIA

Sex neutral, undifferentiated external genitalia start to masculinize at 8-9 weeks of gestation when the potent androgen dihydrotestosterone (DHT) is produced from the testes. DHT is required for fusion of the urethral and labioscrotal folds, lengthening of the genital tubercle, and regression of the urogenital sinus (20). The enzyme 5α-reductase-2 is the enzyme required to convert testosterone produced by the fetal Leydig cells to DHT. Complete masculinization of the external genitalia by DHT is accomplished by week 14 of gestation. In the absence of either testosterone secretion or 5α-reductase-2 activity, the sex neutral external genitalia develop along female lines. Specifically, without DHT the labioscrotal and urethral folds form the labia majora and minora, respectively. The genital tubercle develops into a clitoris and the urogenital sinus gives rise to the urethral opening and anterior portion of the vagina (21). The posterior portion of the vagina develops from the Müllerian ducts in the absence of MIS.

Table 3. Genes and Associated Proteins Involved in Gonadal Differentiation and Associated DSD Presentations in Humans (22, 23)

Gene	Protein/ Function	Presentation
Involved in bipotential gonad		

NR5A1 (Nuclear Receptor Subfamily Subgroup 5 group A member 1)	SF1 (Steroidogenic Factor 1)	XY gonadal dysgenesis with LOF (loss of function)
WT1 (Wilms Tumor 1)	Encodes Zinc finger transcription factor	Denys-Drash syndrome, WAGR, Frasier syndrome
Involved in Testes Development		
DHH (Desert Hedgehog)	Necessary for up-regulation of *SF1* in Sertoli cells	Loss of function: XY gonadal dysgenesis
DMRT1 (Doublesex and MAB3 related Transcription factor)	Required for postnatal maintenance of sertoli and germ cells	Hemizygosity: XY gonadal dysgenesis
GATA4 (GATA binding protein 4)	Transcription factor	Loss of function: XY ambiguous genitalia
NR0B1 (Nuclear receptor Subfamily 0 group B member 1)	Encodes DAX1 (Dosage sensitive sex reversal adrenal, hypoplasia critical region X, chromosome gene 1)	Duplication: XY gonadal dysgenesis and congenital adrenal hypoplasia
SOX3 (SRY-related HMG box 3)	Involved in testes development	Duplication: XX testicular DSD
SOX8 (SRY-related HMG box 8)	Involved in testis-determination	Mutation: XY DSD with streak and female genitalia(24)
SOX9 (SRY-related HMG box 9)	Transcription factor, also involved in chondrocyte differentiation	Loss of function: XY gonadal dysgenesis with campomelic dysplasia GOF: XX female-to- male sex reversal
SOX10 (SRY-related HMG box 10)	Transcription factor	XX masculinized or incompletely feminized
SRY (Sex determining region Y)	Transcription factor	Loss of function: XY ovarian DSD Gain of function: XX testicular DSD
Involved in Ovary Development		

RSPO1 (R-Spondin 1)	Signaling molecule that stabilizes β catenin	XX testicular and ovotesticular DSD Duplication: XY gonadal dysgenesis
WNT4 (Wingless-type MMTV integration site family member 4)	WNT4, a signaling molecule that plays role in müllerian duct formation, oocyte development	Gain of function: XY gonadal dysgenesis Loss of function: XX müllerian duct agenesis, testosterone synthesis

GOF: gain of function, LOF: loss of function

DISORDERS OF SEX DEVELOPMENT (DSD)

The discordance between genetic, gonadal, or anatomic sex is collectively referred to as "disorders of sex development (DSD)". Alternatively, the nomenclature "differences in sex development" is suggested to eliminate the stigma associated with the term "disorders" (25-28). In addition to candidate gene testing, the new era of molecular diagnostic tools including whole exome/genome sequencing has uncovered novel molecular etiologies in the past few years. Accurate molecular diagnosis supplements the management of the affected individuals and provides families with information regarding prognosis and risk of recurrence (25-30). For clinical purposes, DSD (including ambiguous genitalia in newborns) are classified according to the affected individual's karyotype. The consensus statement addressing the approach and care of Disorders of Sex Development (originally published in 2006 and updated in 2016) suggests the following broad classification for DSD: (A) Sex Chromosome DSD, (B) 46, XY DSD, and (C) 46,XX DSD (26, 31) (Table 4).

Table 4. Clinical Classification and Causes of DSD

Sex Chromosome DSD	45,X: Turner syndrome and variants 47,XXY: Klinefelter syndrome and variants 45,X/46,XY: Mixed gonadal dysgenesis and ovotesticular DSD 46,XX/46,XY: Chimeric and ovotesticular DSD
46,XY DSD	Disorders of gonadal development: Complete/partial gonadal dysgenesis, gonadal regression and ovotesticular DSD Defect of Androgen biosynthesis or action: Androgen biosynthesis defects, androgen receptor defects, Luteinizing hormone receptor defects and anti-Mullerian hormone /receptor defect
46,XX DSD	Disorders of gonadal development: Ovotesticular DSD, Testicular DSD and gonadal dysgenesis Disorders of androgen excess: fetal, fetoplacental and maternal Other disorders: Anatomical and syndromic

46,XY DSD with Ambiguous Genitalia

Ambiguous genitalia in a 46, XY newborn can be due to abnormal formation of the early fetal testes (testicular dysgenesis), decreased production of testosterone or dihydrotestosterone (5α-reductase

deficiency), or the inability to respond to androgens (androgen insensitivity syndrome, or AIS). Depending on the degree of androgen production defect or resistance, the phenotype of the external genitals in affected newborns can range from typical female to male appearing external genitalia with a small phallus, hypospadias, and bifid scrotum with or without palpable testes. Only presentations of ambiguous genitalia are considered here. For the purpose of simplicity, we group the underlying causes of 46, XY DSD including ambiguous genitalia according to the following categories: (a) partial gonadal dysgenesis, (b) partial testosterone biosynthetic defects, (c) partial 5α-reductase deficiency, and (d) partial androgen insensitivity syndrome or PAIS.

PARTIAL GONADAL DYSGENESIS

In partial gonadal dysgenesis, it is presumed that a gene mutation results in a partial abnormality in the development of the early urogenital ridge. Partial gonadal dysgenesis could also involve a mutation in genes such as *SRY* and *SOX9,* required for the differentiation of the bipotential gonads into testes. This category of DSD is associated with incomplete masculinization of the external genitalia as well as variable degrees of Wolffian duct maintenance and Müllerian duct inhibition. A clear consensus regarding the gender of rearing for newborns affected by partial gonadal dysgenesis is lacking. Fewer procedures were historically required for surgical feminization (mean = 2.1) compared to surgical masculinization (mean = 5.8) of the genitalia. However, the functional outcome in individuals who received feminizing or masculinizing procedures is less than optimal (32, 33). Additionally, rates of satisfaction with sex of rearing are similar for affected individuals whether raised male or female (1, 26, 31, 34). Further studies are needed to elucidate why some individuals with partial gonadal dysgenesis experience gender dysphoria. Affected individuals are believed to have a high risk for developing gonadoblastomas and malignant transformations. Bilateral gonadectomy is recommended in newborns with this category of 46, XY DSD if their gonads are located in the abdomen and not amenable to monitoring. (35, 36)

Leydig cell aplasia or hypoplasia is a variant of gonadal dysgenesis. It is a condition of inadequate Leydig cell differentiation that results in impaired androgen production. LH receptor defects as well as polymorphism in the LH receptor gene has been described to be associated with this condition (37). The phenotype associated with Leydig cell hypoplasia is incomplete masculinization of the external genitalia accompanied by incomplete development and maintenance of the Wolffian ducts). The risk of developing germ cell tumors in newborns with Leydig cell hypoplasia is unclear, and thus the recommendation for gonadectomy is not well established (1, 31, 37, 38).

DEFICIENCY OF TESTOSTERONE BIOSYNTHESIS

The inability to produce testosterone results from defects in the activity of any of the enzymes required to synthesize testosterone from cholesterol. Identified enzymatic defects include (Figure 1) Steroidogenic Acute Regulatory Protein defect (StAR) , P450 side-chain cleavage deficiency (CYP11A1), 3β-hydroxy steroid dehydrogenase 2 deficiency (HSD3B2), 17α-hydroxylase/17,20-lyase deficiency (CYP17A1), and P450 oxidoreductase deficiency (POR) (39). Additionally, 17β-hydroxysteroid dehydrogenase deficiency (also known as 17β–hydroxysteroid oxidoreductase or 17-ketosteroid reductase, HSD17B3) is a cause of 46, XY DSD (40). P450 oxidoreductase (POR) deficiency has been described to cause ambiguous genitalia in both boys and girls (41-43). Similar to partial gonadal dysgenesis, a partial testosterone biosynthetic defect results in ambiguous external genitalia and variable degrees of Wolffian duct development. However, unlike partial gonadal dysgenesis, Müllerian ducts are not

maintained in 46, XY newborns with partial testosterone biosynthetic defects. The risk of developing germ cell malignancy in newborns with CYP11A1, HSD3B2 or CYP17A1 deficiency is unknown. Newborns affected by 17-ketosteroid reductase deficiency are believed to have a fairly low risk for malignancy (1). If gonads are located in the scrotum, then periodic examination of the testes may be advised as an alternative to gonadectomy (31, 39-43).

5A-REDUCTASE-2 DEFICIENCY

Deficiency of the enzyme 5α-reductase results from mutations in the steroid 5α-reductase type 2 (2p23.1) *SRD5A2* gene that range from single point mutations to entire deletions of the gene. Affected newborns possess fully-functioning Leydig and Sertoli cells, but due to the inability to convert testosterone to DHT, these newborns present with variable degrees of under masculinized external genitalia including genital ambiguity in some cases (21, 44-46). The Müllerian ducts of affected individuals regress as expected due to normal Sertoli cell function. At puberty the testes of affected individuals are capable of spermatogenesis as DHT is not required for germ cell maturation. Therefore, fertility is possible in less severely affected individuals (47-49) or with the use of intrauterine insemination. At puberty, normal testes do not express 5α-reductase activity. Normal virilization at puberty, coupled with fertility potential, are strong factors for recommending male sex of rearing in newborns with this type of 46,XY DSD. Recent evidence suggests that SRD5A2 activity may also be influenced by genetic polymorphisms (50). The risk for germ cell malignancy in affected individuals is unknown and gonadectomy is not currently supported by the literature (1, 31, 46).

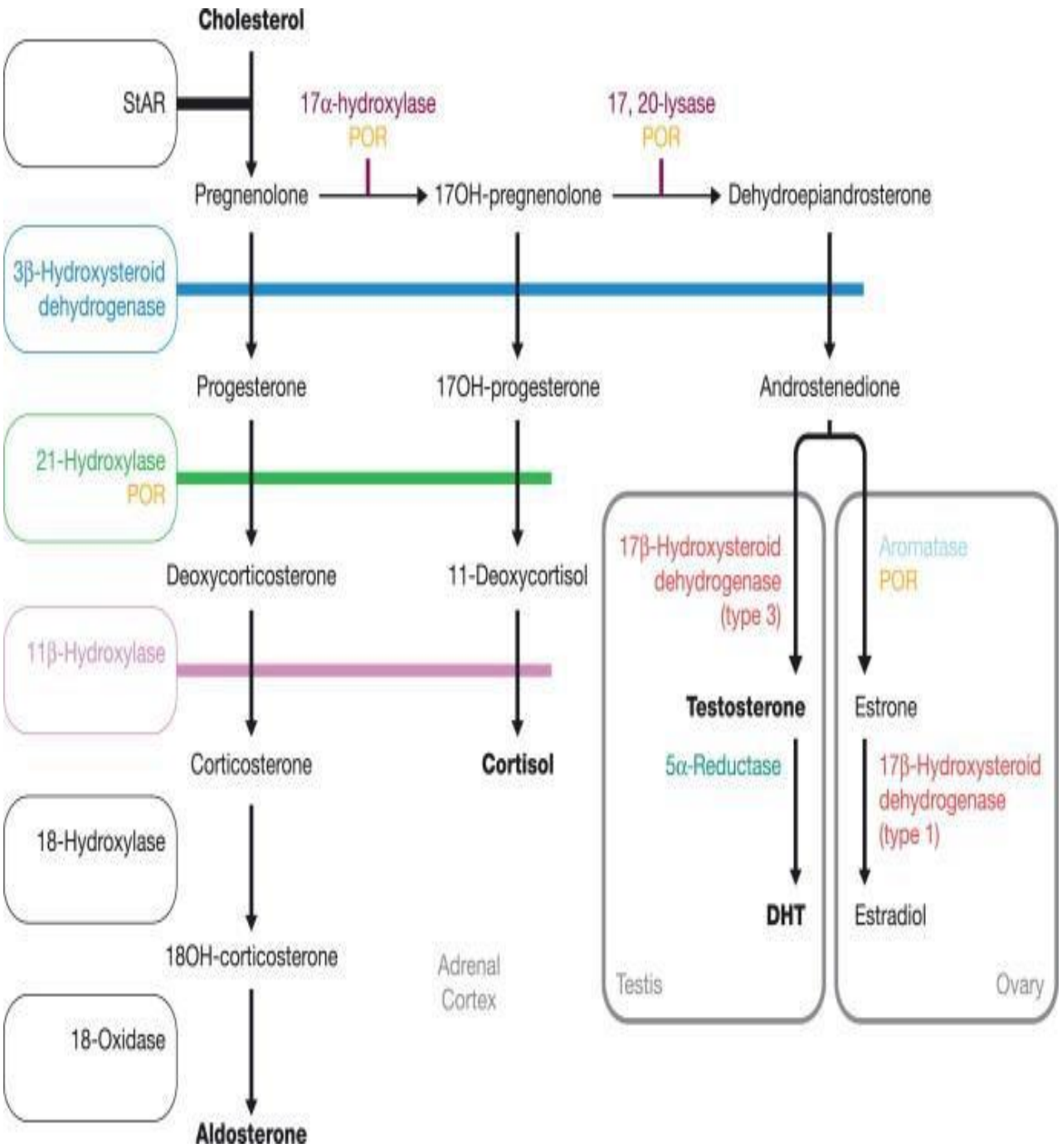

Figure 1. Representation of Steroidogenesis Pathway

ABNORMAL ANDROGEN RECEPTOR ACTIVITY

The human androgen receptor (AR) is encoded by a single gene composed of 8 exons in the q11-12 region of the X chromosome. An AR gene mutation database, updated monthly, includes hundreds of AR lesions that result in varying degrees of atypical sex differentiation of 46, XY fetuses (51-53). A small number of complete AR gene deletions have been reported, as well as deletions starting at exons 2, 3 or 4 and extending to the terminus of the gene. A limited

number of mutations resulting from premature terminations, base deletions and terminations have also been identified. The most common type of AR gene mutation results from base substitutions (54). When mutations result in partial inactivation of the AR, ambiguous genitalia in a person with a 46, XY chromosomal results.

Partial Androgen Insensitivity Syndrome in 46, XY DSD

Individuals with partial androgen insensitivity syndrome (PAIS) experience variable degrees of end-organ unresponsiveness to androgens resulting in variable degrees of Wolffian duct development and external genital ambiguity. Family members with the same androgen receptor gene mutation can express phenotypic variability secondary to variable degrees of insensitivity to androgens (55, 56). Thus, it is difficult to predict if a newborn with PAIS will show a response to future testosterone therapy based on genetic information alone. Similar to partial gonadal dysgenesis, consensus regarding an optimal sex of rearing for newborns affected by PAIS does not currently exist. While fewer procedures are usually required for surgical feminization compared to surgical masculinization in a person with ambiguous external genitalia, it is unclear if the functional outcome is optimal among newborns who receive feminizing procedures compared to those who receive masculinizing procedures. Rates of satisfaction with sex of rearing are similar for individuals with PAIS raised female or male (57). While reports exist of impaired sexual function in people with PAIS raised male, it is highly suspected that sexual functional outcomes in affected people raised female are similarly poor (34). Reports of sexual satisfaction for people with PAIS who have not received genitoplasty are limited; however, more information about these individuals is being added to the medical literature. Newborns with PAIS are considered to be at high risk (50%) for developing gonadal tumors, and bilateral orchiopexy or gonadectomy is recommended at the time of diagnosis if the testes are located in the abdomen (1, 31, 58).

46,XX DSD with Ambiguous Genitalia

A fetus with a 46, XX chromosomal complement and normal ovarian organogenesis can be exposed to excessive amounts of androgens originating either from the fetus itself or from the mother. On occasion, multiple congenital malformations may occur in a 46, XX newborn with ambiguous genitalia.

ABNORMAL FETAL ANDROGEN PRODUCTION: CONGENITAL ADRENAL HYPERPLASIA (CAH)

The term congenital adrenal hyperplasia (CAH) encompasses several adrenal disorders, each related to a mutation of one of the enzymes necessary for the biosynthesis of cortisol from cholesterol (59) (Table 5). These abnormalities result in increased ACTH secretion by the pituitary gland that can in turn result in increased secretion of cortisol precursors including adrenal androgens. Deficiency of steroid acute regulatory protein (StAR) results in congenital lipoid adrenal hyperplasia manifested by salt loss and a lack of cortisol, androgen and estrogen secretion. Genetic female infants affected by StAR deficiency exhibit typical female external genitalia while genetic males are born with ambiguous genitalia.

Deficiency in 17α-hydroxylase/17,20-lyase leads to hypertension due to hypersecretion of corticosterone and impaired androgen secretion. Similar to congenital lipoid adrenal hyperplasia, genetic females affected by 17α-hydroxylase/17, 20-lyase deficiency develop typical female external genitalia while genetic males present with ambiguity. Deficiency of 3β-hydroxysteroid dehydrogenase results in salt loss and impaired androgen synthesis. Affected genetic females exhibit minimal genital masculinization while genetic males are variably

under masculinized (60).

CAH due to 11β-hydroxylase deficiency and 21-hydroxylase deficiency results in the most pronounced masculinization of external genitalia in genetic females of all of the types of CAH. Additionally, 11β-hydroxylase deficiency leads to hypertension in either sex (61, 62). CAH due to 21-hydroxylase deficiency represents the most common form (more than 90% of cases) of CAH (59, 63). In the milder (simple-virilizing) presentation of classical 21-hydroxylase deficiency, salt loss is typically not a problem, while in the more severe (salt-wasting) form of classical 21-hydroxylase deficiency this does occur (59, 63, 64).

Table 5. Human Adrenal Steroidogenic Enzymes and the Effects of Their Deficient Production on Genital Phenotypes in 46, XY and 46, XX Fetuses

Enzyme	Gene/Locus	Genital Phenotype in a 46,XY Fetus	Genital Phenotype in a 46,XX Fetus	Additional Features
StAR	*STAR/8p11.2*	Ambiguous	Normal	Adrenal failure/pubertal failure
P450 scc	*CYP11A1/15 q23-q24*	Ambiguous	Normal	Adrenal failure/ pubertal
3βHSD	*HSD3B2/ 1p13.1*	Ambiguous	Clitoromegaly	Primary adrenal failure
P450c17	*CYP17/10q24.3*	Ambiguous	Normal	Hypertension
P450 Oxido-reductase	*POR/7q11.2*	Ambiguous	Ambiguous	Sometimes associated with Antley-Bixler syndrome
P450c11 β	*CYP11B1/8q21-q22*	Normal	Ambiguous	Hypertension
P450c21	*CYP21A2/6p21-23*	Normal	Ambiguous	Variable degree of adrenal failure

EXCESS MATERNAL ANDROGEN PRODUCTION

Because excessive androgen production adversely affects fertility due to anovulation, cases of maternal androgen production during pregnancy are extremely rare. That said, maternally-derived androgens can cross the placenta to masculinize a female fetus. The origin of these maternal androgens is usually the ovaries or adrenal glands. Androgen-producing tumors of the ovary include hilar cell tumors,

arrhenoblastomas, lipoid cell tumors, and Krukenberg tumors. Androgen secreting tumors of the adrenals also occur, albeit rarely, during pregnancy (65).

PLACENTAL AROMATASE DEFICIENCY

During fetal development the adrenals produce large amounts of 17-hydroxypregnenolone and 16-hydroxy-DHA. These steroids are transferred to the placenta and then converted into androgens and then estrogens. If an aromatase enzyme deficiency exists, the androgen precursors accumulate and return to fetal circulation resulting in masculinization of female fetuses (66).

DRUGS ADMINISTERED TO THE MOTHER DURING GESTATION

In 1958 Wilkins et al. reported that some synthetic progestins administered to pregnant women such as 17α-ethinyl-19-nortestosterone masculinize the external genitalia of female fetuses (67, 68). The use of androgenic progestins is now avoided in pregnancy.

Other Categories of Ambiguous Genitalia

SYNDROMES ASSOCIATED WITH MULTIPLE CONGENITAL ANOMALIES

Ambiguous genitalia can be associated with syndromes of multiple congenital malformations (69). These associations are due to the fact that many of the transcription factors involved in sex development and differentiation are also involved in other developmental functions. For example, mutations of the *WT-1* gene (11p3) can result in a number of syndromes including WAGR, Denys-Drash or Frasier syndromes (70-72). *SOX-9* (17q24-25) mutations can result in campomelic dysplasia (73). Mutations of *DMRT1/DMRT2* (9p24.3), *EMX-2* (10q25.3q26.13), *ATRX* (Xq13.3) and *WNT-4* (1p35) are often associated with developmental delay (74, 75). *SF-1* (9q33) and *DAX-1* (Xp21.3) play roles in early formation of the adrenals, anterior pituitary and parts of the hypothalamus. Thus, mutations result in abnormalities of these organs in addition to those of the urogenital system. Abnormal development of the lower abdominal wall with pubic diastesis results in urogenital anomalies in the VATER and CHARGE syndromes, as well as bladder and cloacal exstrophy (76). These examples demonstrate the complexities of the anomalies that can be associated with ambiguous genitalia in newborns.

OVOTESTICULAR DSD

Ovotesticular DSD occurs when both ovarian and testicular tissue develop in the same individual (1, 31). Most newborns with ovotesticular DSD possess a 46, XX chromosome complement and present with ambiguous genitalia; however, some affected individuals possess a 46, XY chromosome complement or 46, XX/46, XY mosaicism. Similar to 46, XY DSD, the degree of testicular development will dictate the degree of masculinization of the external genitalia, extent of Wolffian duct development and Müllerian duct regression in affected newborns (54). In newborns with ovotesticular DSD the risk of germ cell tumors is believed to be low (3%) (1, 31).

WORK-UP OF NEWBORNS WITH AMBIGUOUS GENITALIA

The etiology underlying genital ambiguity in newborns impacts several aspects of management including recommendations for sex of rearing, assessing risk for gonadal malignancy, and the need to replace hormones such as cortisol, aldosterone and/or steroid hormones. Thus, it is important to initiate an appropriate work-up expeditiously.

Clinical Review and Evaluation

Look for signs of dehydration including vomiting and diarrhea, as these are symptoms of a salt-losing crisis for newborns unable to produce sufficient amounts of the hormone aldosterone. A careful examination of the external genitalia should also be performed as this is a non-invasive, albeit indirect, bioassay of prenatal exposure and response to DHT as well as the timing of androgen exposure.

Androgen exposure needs to occur early in the first trimester to cause labial fusion. A genital exam should include a measure of stretched phallic length, evaluation of the quality of the corpora, and inspection of the labia, labio-scrotal folds or scrotum. The position of the urethral opening (and vaginal opening if applicable) should be documented, as well as the presence and location of palpable gonads when appropriate. Asymmetry of the gonads and genitalia should be evaluated. Finally, anogenital distance can be helpful in determining the amount of androgen exposure experienced during fetal development (a higher ratio is consistent with increased androgen exposure).

Status of Body Hydration

Serum electrolytes and glucose levels should be monitored daily as cortisol deficiency can manifest as hypoglycemia in newborns affected by CAH. Body weight should also be monitored as excessive weight loss may indicate pathological dehydration.

Karyotype

It is crucial to obtain a karyotype as soon after birth as possible. Laboratories should be informed to look for sex chromosome mosaicism and deletions. Studies of the fluorescence of the long arm of the Y chromosome and hybridization with an SRY gene probe on the short arm of the Y chromosome (FISH) are also helpful.

Hormone Studies

Detailed hormone studies may be indicated including serum gonadotropins (LH, FSH), androgens and androgen precursors (17-hydroxypregnenolone, 17-hydroxyprogesterone, androstenedione, testosterone, dihydrotestosterone), adrenal steroids (cortisol, aldosterone, and their precursors), and Müllerian inhibiting substance (MIS) (30, 31). Care must be taken when interpreting the results in premature babies, in whom these studies may need to be repeated at a later age.

Imaging Studies

A sonogram or MRI can be helpful in identifying both the type and extent of internal sex organ development. Imaging can also detect abnormalities of the urinary tract (kidney, ureters, bladder) that sometimes occurs in conjunction with genital ambiguity. An MRI may be better able to identify Müllerian structures (uterus, fallopian tubes, upper portion of the vagina) than a sonogram, and can also be useful for localizing abdominal gonads. A genitogram can be used for visualization of the urinary tract, and to determine its position in relation to the vagina or vagino-utricular pouch, when present. However, cystoscopy and vaginoscopy are usually preferred to genitogram, especially in high volume surgical centers, due to better visualization of anatomy immediately prior to surgery without a separate pre-operative procedure (77).

Molecular Studies

As our knowledge of the genes involved in sex differentiation increases, and as molecular technologies develop and become more accessible, it will be possible to better determine specific genetic etiologies underlying DSD in the future. Some genetic investigations, such as detection of AR gene mutations, are already available clinically. Recently,

techniques that look at several candidate genes, such as massively parallel sequencing (MPS) or whole exome/genome sequencing (WES, WGS) have come to the forefront. These genetic panels may provide more definitive answers, especially for patients with 46, XY DSD in which the etiology is unclear (30, 78, 79).

DIFFERENTIAL DIAGNOSIS

Results from the karyotype will play a major role in the differential diagnosis as the nomenclature for DSD is based primarily on genetic sex. In most cases, the chromosome complement will be either 46,XX or 46,XY. In rare instances, the chromosome complement will be 45,X/46,XY or 46,XX/46,XY mosaicism.

46, XX DSD

A 46, XX karyotype in a newborn with ambiguous genitalia indicates that the child is a genetic female who was exposed to excessive amounts of androgens during fetal life. Marked elevation of plasma 17- hydroxypregnenolone, 17-hydroxyprogesterone and androstenedione, along with male-typical levels of testosterone, are characteristic of 21-hydroxylase deficiency. High values of corticosterone and 11- deoxycortisol, along with elevated androgens, indicate 11β-hydroxylase deficiency.

When excess maternal androgen production is the underlying cause for masculinization of a female fetus, the source of these steroids is eliminated postnatally. Thus, the various steroids studied in affected newborns will be in the female-typical range despite masculinization of the external genitalia.

In individuals with ovotesticular DSD including a 46, XX chromosome complement, masculinization arises from androgens secreted by the testicular portion of the differentiated gonads. Androgen production is similar to that produced by testes in unaffected males except that the amount is usually smaller. The degree of masculinization of the genitalia is thus related to the amount of functioning testicular tissue present.

On occasion, translocation of the pseudo-autosomal part of the Y chromosome along with a mutated SRY gene to an X chromosome occurs. The result is partial masculinization of the genitalia in a 46,XX newborn. With maturity, the phenotype of affected individuals closely resembles that of boys and men with Klinefelter syndrome.

Very low values of MIS are expected in 46, XX newborns with masculinized genitalia that is attributed to CAH or excess maternal androgen production during gestation. MIS is higher in newborns with ovotesticular DSD, due to Sertoli cell development in the testicular portion of their gonads.

46, XY DSD

A 46, XY karyotype reveals that one is dealing with a genetic male who was under-masculinized during fetal development. Laboratory findings of normal or elevated testosterone and DHT indicate a diagnosis of AIS. If testosterone levels are normal but DHT levels are low, a diagnosis of steroid 5α-reductase deficiency can be made. Low levels of testosterone and DHT, along with marked elevation of some androgen precursors, indicates a deficiency of one of the enzymes required for androgen biosynthesis. For example, if the elevated precursors include androstenedione and 17-hydroxyprogesterone, then the defective enzyme is 17-ketosteroid reductase. In all cases of testosterone biosynthetic defects, MIS levels are similar to those observed in male infants not affected by DSD. Finally, when all androgens and their precursors are below normal levels one is dealing with either gonadal dysgenesis or 46, XY ovotesticular DSD. In these cases, MIS values

should also be low. In contrast, for babies affected by Leydig cell hypoplasia, androgens and their precursors are low, while MIS values should be in the normal male range. In patients with 46, XY DSD, it is also important to appreciate the risk for tumors in testes located in the abdomen. The consensus statement on management of DSD has outlined recommendation on timing of gonadectomy for some patients. (1)

SEX OF REARING

Whether or not the etiology of the genital ambiguity can be determined, a sex of rearing should be elected for a newborn with ambiguous genitalia (1). Ideally, we believe that the sex of rearing for newborns who present with ambiguous genitalia should be decided as early as possible, but only following an appropriate work-up with time provided to the parents to consider all of their options. Gender reassignment later in childhood can be difficult for both the child and the child's family (80, 81). We also believe that the final choice of gender assignment should be made by parents after receiving recommendations and education from the medical team. The role of this team (pediatric endocrinologist, pediatric surgeon, geneticist, psychologist and others) is to inform parents about the process of sex determination and differentiation, and the specific cause for DSD (when known) that affects their child (1). As knowledge concerning the long-term outcome of people affected by DSD accrues, this too must be shared with parents (1, 4, 5, 82-96). The following recommendations for assigning a sex of rearing apply to infants who present as newborns with ambiguous genitalia (1).

Gender Assignment for Newborns Affected by 46, XX DSD

As noted earlier, masculinization of newborns with a 46, XX chromosomal complement is usually a result of CAH and rarely results from exposure to androgenic substances transferred from the mother to the fetus via the placenta. The most common etiology of ambiguous genitalia in genetic females is CAH due to 21-hydroxylase deficiency (64). Masculinization due to 21-hydroxylase deficiency does not impair the development of the ovaries or the Müllerian ducts. Historically, female sex of rearing has been recommended for this group as most develop a female gender identity (86-88) and female assignment allows for future fertility potential. However, actual fertility rates in women affected by 21-hydroxylase deficiency are low (96). Limited information is available about the medical and psychosexual outcome of 46,XX newborns affected by 21-hydroxylase deficiency and raised male (97, 98), although these patients do tend to have an increased risk of gender dysphoria compared to their female counterparts (99).Thus, a female gender of rearing is typically the decision for 46,XX newborns affected by 21-hydroxylase deficiency (97). Female gender of rearing is also recommended for conditions resulting from maternal overexposure to androgens and placental aromatase deficiency.

Gender Assignment for Newborns Affected by 46, XY DSD

Generally, people believe that the sex chromosomes indicate a person's "true sex" and laws exist, to the detriment of some individuals affected by DSD, to support this idea (100). Scientifically speaking, it is clear that the majority of genes on the X chromosome do not influence sex development and differentiation, although the AR gene is necessary for phenotypic masculinization. Concerning the Y chromosome, only the *SRY* gene contributes to testicular formation. In fact, most of the genes required for sex development and differentiation are found on the autosomes (1). As such, a 46, XY chromosomal complement does not necessarily dictate a male gender of rearing in newborns affected by 46, XY DSD.

Newborns presenting with a 46, XY chromosome complement and female external genitalia due to complete androgen insensitivity syndrome (CAIS), complete gonadal dysgenesis (Swyer syndrome), or other complete abnormality of testosterone biosynthesis live successfully when assigned a female sex of rearing. Female assignment in such cases is widely accepted by patients throughout their lives despite challenges that they may experience regarding sexual dysfunction and infertility (1, 57, 82, 89).

A category of 46, XY DSD in which male rearing is preferable is in newborns affected by 5α-reductase-2 or 17β-hydroxysteroid dehydrogenase deficiency (1, 45, 92, 93). At puberty, people with these conditions secrete normal amounts of testosterone and masculinization results. In both conditions gender identity
and role frequently changes toward male in patients reared female (101). Additionally, future fertility potential exists for newborns raised male (1, 85).

The most difficult 46, XY DSD patients, in terms of recommending a sex of rearing, are those newborns with ambiguous external genitalia including a small phallus and perineo-scrotal hypospadias (55, 94, 95). In these cases, genital surgeries are often opted for by parents regardless of gender of rearing. Evidence-based information pertaining to satisfaction with sex of rearing, number and type of surgical procedures anticipated for male versus female assignment, future sexual function and the possibility for future fertility needs to be collected including information on children for whom parents chose to not receive genital surgery (1). Regarding satisfaction with sex of rearing, either male or female assignment is possible (55, 94, 95). In terms of the number of genital surgeries performed, female sex of rearing typically requires fewer procedures (59). People with 46, XY DSD report some problems with long-term sexual function whether they are raised male or female (44, 54, 55, 102). People affected by 46, XY DSD and reared female reported a similar degree of dissatisfaction with both the appearance and function of their genitalia compared to and surgical treatment for newborns with ambiguous genitalia, including a better understanding of outcomes for people who do not receive genitoplasty.

PARENTS AND CAREGIVERS OF CHILDREN WITH AMBIGUOUS GENITALIA

Only recently has the understanding of, and reaction to, having a child with ambiguous genitalia received systematic study. For some parents and caregivers, feelings of isolation and concern over what the future may hold for their affected child in terms of stigmatization and sexual dysfunction are paramount (103). One potential approach to ameliorate this issue is to provide information and management options to the family early in pregnancy. Research has been done to look at prenatal diagnosis and treatment of females with 21-hydroxylase deficiency CAH, one of the most common causes of ambiguous genitalia. Doses of dexamethasone started at or before the ninth week of pregnancy and continued until term have been shown to reduce virilization at a rate of 80-85% (104). However, treatment of these fetuses has been controversial due to several reasons. Most notably, only 1 of every 8 patients is an affected female that would benefit from treatment, and invasive methods for determining sex and CAH status (which also carry risk) are not available before the treatment window for dexamethasone is open. This leads to unnecessary treatment for several weeks until these tests can confirm the status of CAH. Now, however, noninvasive prenatal diagnosis using massive parallel sequencing of cell-free fetal DNA may be able to determine the prenatal diagnosis as early as 6 weeks of gestation(104, 105). Further studies need to be done on this subject, but for families who already have an affected child or know that they are carriers, this may be an option in the future. In addition, these tests could be used to look at other autosomal dominant or X-linked

conditions that cause ambiguous genitalia, especially when the fetus is known to be at risk(105).

Patients for whom prenatal diagnosis and/or treatment is not an option, prognosis and management has improved significantly over time but many complexities still exist. Our studies of parents of children with DSD reveal that the appearance of atypical genitalia can result in significant stress and maladaptive parenting strategies. For example, mothers of children with ambiguous genitalia experience greater stress if their child has not received "corrective" genital surgery(106) and some parents believe such surgeries eliminate stigmatization that may arise due to their child's ambiguous genitalia (103). However, as their children mature past infancy, some parents realize that their child's DSD has not been ameliorated by genitoplasty, and concerns for their son or daughter resurface (103). To illustrate, among parents studied by our group, caregivers of adolescents with DSD experienced increased stress as their child matured despite the fact that many had received earlier surgery (107). Additionally, parents of children reared male reported the most perceived child vulnerability for their sons (108) and their reported levels of depression were directly associated with more atypical genital appearance for their sons (109). In summary, parents report that while early genitoplasty seems to "fix" some of their concerns for their child with DSD, this fix is not long-lasting. Instead, what needs to be emphasized to families is how to support their child who is different, but not damaged. Family centered, interdisciplinary care with open and clear communication between patients, parents, and caregivers has been shown to be essential for optimal quality of life (101).

In addition, networks of families who have the personal experience of parenting children with ambiguous genitalia can be an invaluable resource (110).

SUMMARY

Newborns who present with ambiguous genitalia must be considered medical emergencies due to the life-threatening issues present in some cases. A work-up should be started immediately in the attempt to obtain a precise diagnosis when possible. In 46, XX DSD, female gender of rearing is typically observed as most cases will develop the potential for fertility despite masculinization of the external genitalia.

Additionally, much evidence indicates that development of female gender identity occurs in those patients. In 46, XY DSD, female or male rearing may be appropriate. Male sex of rearing is recommended for patients with 5α-reductase or 17β-hydroxysteroid dehydrogenase deficiency due to male fertility potential for these groups. Following a proper diagnosis, parents must be educated about DSD, including what is known about long-term outcomes. Once parents have received this education, the medical team should support the parents as they decide on an appropriate sex of rearing for their affected newborn. Finally, referring parents to support groups and introducing them to other caregivers of children born with ambiguous genitalia is extremely important to optimize parents' understanding and acceptance of their child's condition. With increased understanding and acceptance, optimal growth and development for children born with ambiguous genitalia may be obtained.

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