Human sex differentiation and its abnormalities

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The purpose of this chapter is to review the presentation and management of patients affected by conditions of abnormal sex differentiation. First, the processes of normal sex differentiation are covered, followed by an overview of the various syndromes of abnormal sex differentiation, or intersex conditions, that can occur. These disorders are presented according to the following categories: patients who possess a 46,XX chromosome complement, those who possess a 46,XY chromosome complement, and individuals who present with an atypical sex chromosome complement (i.e. 45,XO or 45,XO/46,XY mosaicism). A description of the medical, surgical and psychological treatment options for people affected by various intersex conditions and reared as females are included. Practice points, based on research studies when available, are dispersed throughout the chapter. Additionally, information pertaining to relevant Internet websites and patient support groups are provided, so that medical staff can educate their patients about the availability of these resources.

Key words: sex; gender; sex differentiation disorders; sex chromosome abnormalities; genes; gonads; sex hormones; genitalia; intersex.

PHYSIOLOGY OF NORMAL SEX DIFFERENTIATION

Sex is multidimensional. By this, we mean that no single gene, hormone, anatomical feature or behaviour indisputably determines the sex of an individual (www.hopkinsmedicine.org/pediatricendocrinology/intersex). The present section provides a general review of the physiology of normal sex differentiation. Included is a consideration of the genetic and hormonal influences on the anatomical components of sex (e.g. gonads, internal sex ducts, external genitalia). Finally, we consider gender, a behavioural component of an individual's sex, as it relates sex chromosomes, gonads, sex steroid hormones, sex ducts and external genitalia.
Genes

The first step in sex differentiation is the union of an egg (23,X chromosome complement) with a sperm (23,X or 23,Y chromosome complement). Males possess a 46,XY chromosome complement and females possess a 46,XX chromosome complement. During the first weeks of development, all embryos are phenotypically similar regarding sex differentiation (e.g. the appearance of the bipotential gonads, the presence of both Müllerian and Wolffian ducts, the appearance of the bipotential external genitalia) despite the chromosome complement.

The sex-determining region of the Y chromosome, SRY, is one of the genes required for bipotential gonads to differentiate into testes. Since the identification of SRY, a number of transcription factors (e.g. DAX-1, SF-1, WT-1, SOX-9, DMRT1/DMRT2) have also been recognized for their role in the differentiation of bipotential gonads into either testes or ovaries. Many additional, but still unknown, gene products are probably involved in sex differentiation.

Practice points

- testing for the presence of the SRY gene, via fluorescence in situ hybridization (FISH), is a fast and reliable way to determine the genetic sex of a child for whom abnormal sex differentiation is suspected

Research agenda

- identification of additional transcription factors, as well as the interaction of these genes; this is important for the process of gonadal differentiation

Gonads

The gonadal ridge, or the precursor to the bipotential gonads, develops during the first weeks of gestation. This structure, along with the primordial germ cells, in turn becomes a bipotential gonad. The differentiation of bipotential gonads into testes in cases of a 46,XY chromosome complement, or into ovaries in cases of a 46,XX chromosome complement, is the typical path of early gonadal sex development.

Hormones

Three types of cell differentiate in the testes: Sertoli cells, Leydig cells and spermatogonia. The Sertoli cells produce the hormone Müllerian inhibiting substance MIS.

Practice points

- MIS is produced by fetal testes throughout gestation and is detectable until puberty. MIS is also produced by fetal ovaries, but not until after the Müllerian ducts have been established. At birth, MIS levels can be used as an indicator of the extent of Sertoli cell development in an infant whose gonadal development is in question
(MIS) and the Leydig cells produce testosterone, both needed for normal male fetal development to proceed. Three types of cells also differentiate in the ovaries; follicular cells, steroid-producing cells and oocytes. In contrast to the testes, the ovaries are not thought to produce significant amounts of steroids prior to puberty.

Internal ducts

Both Müllerian and Wolffian ducts develop in all fetuses, regardless of genetic or gonadal sex, during early gestation. In males, the Müllerian ducts regress in response to MIS produced by the Sertoli cells, and the Wolffian ducts develop in response to production of testosterone by the Leydig cells. In contrast, the Müllerian ducts develop in females in the absence of MIS, and the Wolffian ducts fail to develop in the absence of testosterone production.

External genitalia

Like the bipotential gonads, the external genitalia are initially identical in all fetuses, regardless of the genetic or gonadal sex. The external genitalia can develop along either male or female lines. If dihydrotestosterone (DHT) is produced in sufficient amounts from gestational weeks 7–8 until birth, and if the fetus can respond normally to androgens, then the bipotential genitalia will develop in a male-typical manner (e.g. the genital tubercle develops into a penis, the urethral fold fuses so that the opening is located at the tip of the penis, and the genital swelling fuses to form a scrotum).

In contrast to the fetal testes, the fetal ovaries do not produce androgens that masculinize the external genitalia. In the absence of androgenic effects, the genital tubercle forms a clitoris, the urethral fold develops into the labia minora, the urethral
opening is located on the perineum, and the genital swelling forms the labia majora. Finally, in the absence of MIS production, a normal vagina is formed.

Gender

The final component to a person’s sex is their behavioural sex, or gender. Gender is a broad term that encompasses how a person views oneself as a man or woman (gender identity or GI), how that person is viewed by other members of society as masculine or feminine (gender role or GR), and their erotic behaviour.10,11
either over-masculinization in subjects with a 46,XX chromosome complement (also referred to as female pseudohermaphroditism), or under-masculinization in subjects with a 46,XY chromosome complement (also referred to as male pseudohermaphroditism). It is also necessary to consider abnormal sex differentiation in patients with sex chromosome abnormalities such as Turner syndrome (45,XO).

**Abnormal sex differentiation in patients with a 46,XX chromosome complement**

Most patients with ambiguous genitalia and a 46,XX chromosome complement are affected by CAH (Table 1). In these girls and women, the gonads develop into ovaries, the Mullerian ducts are present, and the Wolffian ducts are regressed. In rare cases of female pseudohermaphroditism not related to CAH, the bipotential gonad does not completely differentiate into ovaries. Such a situation can occur with true hermaphroditism or 46,XX gonadal dysgenesis. The degree of atypical ovarian differentiation associated with true hermaphroditism or gonadal dysgenesis can vary widely. Finally, in rare instances, masculinization of female fetuses can occur as a result of excessive production of maternal androgen.

**Practice points**
- the first step for correct identification of an intersex condition is to obtain a karyotype
- in the majority of cases of ambiguous genitalia in patients with a 46,XX chromosome complement, the aetiology of the over-masculinized genitalia is due to CAH resulting from 21-hydroxylase deficiency. In newborns, the following laboratory tests can be used to make a diagnosis of CAH resulting from 21-hydroxylase deficiency: androgens and androgen precursors (testosterone, DHT, 17-hydroxyprogesterone), cortisol and aldosterone. Serum electrolytes and glucose should also be monitored

**21-Hydroxylase deficiency**

21-Hydroxylase (21-OH) deficiency results in the most frequent form of CAH. 21-OH deficiency is characterized by an inability to produce cortisol, resulting in increased secretion of ACTH and androgens. The major adrenal androgens

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**Table 1. Classification of abnormal sex differentiation in 46,XX subjects.**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital adrenal hyperplasia (CAH)</td>
<td>21-Hydroxylase (CYP21) deficiency</td>
</tr>
<tr>
<td>Excessive maternal androgens</td>
<td>11β-Hydroxylase (CYP11B1) deficiency</td>
</tr>
<tr>
<td>46,XX Gonadal dysgenesis</td>
<td>3β-Hydroxysteroid dehydrogenase (3β-HSD2) deficiency</td>
</tr>
<tr>
<td>46,XX True hermaphroditism</td>
<td>iatrogenic virilizing tumour of adrenal or ovary</td>
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are androstenedione and dehydroisoandrosterone. Both hormones can be metabolized into testosterone and DHT, resulting in masculinization of a female fetus. The precursors of cortisol include 17α-hydroxyprogesterone, progesterone and 16α-hydroxyprogesterone. When secreted in large amounts, these progestins create a salt-losing tendency. If the 21-OH mutation is not severe, the adrenal gland can compensate for this salt loss with increased production of aldosterone (referred to as the simple-virilizing or non-salt-losing form). When the 21-OH mutation is more severe, the result is little aldosterone compensation for salt loss. This scenario is referred to as the salt-losing form of CAH (www.hopkinsmedicine.org/pediatricendocrinology/cah). CAH due to 21-OH deficiency is an autosomal recessive condition. Heterozygous mutations of the 21-OH gene occur in approximately one in 60 people, and the frequency of the homozygous expression of CAH is one in 14 000 Caucasian and Hispanic births, and one in 45 000 African American births.

Mild forms of 21-OH deficiency, referred to as attenuated or non-classical forms of CAH, can present as virilization in childhood. This form of 21-OH deficiency occurs in about one in 35 000 individuals, but is usually identified only in affected females. In some cases, symptoms are not evident until puberty. Attenuated 21-OH deficiency can present as hirsutism and amenorrhoea in adult women as well. Some ethnic groups (e.g. Ashkenazi Jews or people of Mediterranean descent) are more frequently affected than others by the attenuated form of CAH. This situation can probably be attributed to a founder’s effect.

11-Hydroxylase deficiency

This deficiency represents approximately 5% of CAH cases and occurs in approximately one in 100 000 births. The gene needed to produce 11-hydroxylase is CYP11B1. Homozygous mutations of this gene result in the inability to synthesize cortisol and aldosterone. Corticosterone, a precursor to cortisol, and 11-deoxycorticosterone, a salt-retaining hormone, are secreted in large amounts in patients affected by 11-hydroxylase deficiency. The result of hypersecretion of these hormones is hypertension. Thus, 11-hydroxylase deficiency is also known as the hypertensive form of CAH. Additionally, increased production of adrenal androgens, similar to the case of 21-OH deficiency, can result in masculinization of females during fetal development.

3β-Hydroxysteroid dehydrogenase deficiency

The 3β-hydroxysteroid dehydrogenase deficiency is the least frequent form of CAH, representing less than 2% of cases. Individuals affected by 3β-hydroxysteroid dehydrogenase deficiency exhibit an inability to synthesize both cortisol and aldosterone. Therefore, 3β-hydroxysteroid dehydrogenase deficiency represents a salt-losing form of CAH. The androgen dehydroisoandrosterone is produced in large amounts, but affected females are not severely masculinized during fetal life due to the weak androgenic actions of this androgen. Activity of 3β-hydroxysteroid dehydrogenase is also necessary for the production of testosterone by the testes. Therefore, 3β-hydroxysteroid dehydrogenase deficiency results in a lack of testosterone production in males. Such a situation results in genital ambiguity in affected males. Although severe forms of 3β-hydroxysteroid dehydrogenase deficiency are rarely observed in infants, it has been suggested that milder forms are identified more often in adolescent girls and women, resulting in hirsutism and amenorrhoea.
Excessive maternal androgens

In the past, some of the synthetic progestins prescribed to women who experienced habitual abortions exerted some androgenic activity and were subsequently found to masculinize the external genitalia of females during fetal development. Presently prescribed progestins do not have such strong androgenic effects. Thus, cases of iatrogenic masculinization in females attributed to maternal ingestion of hormones are now considered to be rare. Excessive endogenous androgen production (i.e. from ovarian, adrenal or Krukenberg tumours) is not conducive to pregnancy; however, on occasion these hormones can cross the placenta and the result is masculinization of the female fetus.

46,XX True hermaphroditism and 46,XX gonadal dysgenesis

True hermaphroditism refers to the condition in which well-defined testicular tubules occur along with well-defined ovarian follicles in the same individual. Affected individuals may have one gonad that is a testis and the other that is an ovary, or they may possess a combination of ovarian and testicular elements within the same gonad. Most patients with true hermaphroditism have a 46,XX chromosome complement; however, 46,XY and mosaic 45,X0,46,XY chromosome complements are also associated with true hermaphroditism. Although testicular development is common in 46,XY individuals, it is difficult to explain the presence of testicular tissue in 46,XX patients with true hermaphroditism. It has been suggested that these difficult-to-explain cases are related to an autosomal mutation. The extent of masculinization of the external genitalia and the extent of development of the Wolffian ducts will depend on the amount of testosterone that is secreted by the testicular portions of the gonads.

In 46,XX gonadal dysgenesis, patients present entirely female, but fail to develop into a normal female puberty. These patients have elevated gonadotropins and streak gonads. These streak gonads are similar to those found in patients with Turner syndrome (45,XO chromosome complement), discussed later in this chapter. The main difference between 46,XX gonadal dysgenesis and Turner syndrome is that the latter can be associated with multiple congenital malformations, including short stature.

Abnormal sex differentiation in patients with a 46,XY chromosome complement

For infants affected by abnormal sex differentiation who possess a 46,XY chromosome complement, under-masculinization may be attributed to a variety of problems. For example, the early bipotential gonads may not differentiate into testes, with the result that both androgen and MIS production is impaired. In other cases, the testes may be unable to produce testosterone or convert testosterone to DHT, while the production of MIS is normal. Alternatively, the fetal testes produce sufficient amounts of both androgens and MIS, accompanied by decreased androgen receptor activity. With dysfunctional Sertoli cells, MIS production may be impaired while androgen production and responsiveness are normal. Finally, true hermaphroditism can be observed in patients with a 46,XY chromosome complement as well as in those with a 46,XX chromosome complement (Table 2).
Abnormalities of early gonadal development

In the early stages of fetal development, cells organize into a bipotential gonad under the influence of many transcription factors including, but not limited to, SRY, SF-1, WT1, SOX9, DMRT1/DMRT2, DAX-1, and WnT4. The differentiation of a bipotential gonad into a testis also requires the action of several of these same transcription factors. The mutation of some of these factors can be associated with extragonadal abnormalities: SF-1 mutations with hypofunction of the hypothalamus, WT-1 mutations with Denys–Drash and Fraisier syndromes, and SOX-9 with campomelic dysplasia. Abnormalities of any of these genes can result in either complete gonadal dysgenesis (Swyers syndrome) or in partial gonadal dysgenesis. By definition, gonadal dysgenesis is a deficiency of both Leydig cell and Sertoli cell development and function.

In complete gonadal dysgenesis, a total lack of Leydig cells and thus a complete inability to produce testicular androgens results in external genitalia with a normal female appearance. Additionally, in the absence of Sertoli cell development and MIS production, the Müllerian ducts persist. In partial gonadal dysgenesis, partial masculinization of the external genitalia and partial development of the Wolffian ducts result from limited production of testicular androgens and MIS. The degree of abnormal development is directly related to the degree of dysgenesis of the gonads.

Abnormal Leydig cell function

The Leydig cells of the testes synthesize testosterone from cholesterol. The enzymes needed for the biosynthesis of testosterone are the steroidogenic acute regulatory proteins (StAR) and CYP17, which catalyze the conversion of cholesterol to pregnenolone and then to progesterone, respectively. StAR transports cholesterol from the outer to the inner mitochondrial membrane, allowing it to be used by CYP17 in the synthesis of pregnenolone. The StAR gene is highly expressed in Leydig cells and mutations in this gene can lead to Leydig cell hypoplasia.

| Abnormalities of early gonadal development | SRY, SF-1, WT1, SOX9, DMRT1/DMRT2, DAX-1, WnT4 |
| Abnormalities of Leydig cell function | Genes for LHRH, LHRH receptor, LH, LH receptor |
| StAR-Reductase deficiency | S6AR, CYP11A, 3β-HSD2, CYP17, 17β-HSD3 |
| Androgen insensitivity syndrome (AIS) [complete (CAIS) or partial (PAIS)] | Androgen receptor (AR) |
| Isolated persistence of Müllerian ducts | MIS or MIS receptors I and II |
| 46,XY True hermaphroditism | SRY |

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enzyme (StAR), 20-hydroxylase, 22-hydroxylase/20,22-desmolase, side-chain cleavage
enzyme (CYP11A), 3β-hydroxysteroid dehydrogenase (3β-HDS2) and 17-hydroxylase/17,20 desmolase (CYP17).4

A complete deficiency of StAR results in an inability to produce any gonadal or adrenal steroids. This form of pseudohermaphroditism is termed lipoid adrenal hyperplasia.15 The resulting total absence of androgen secretion results in female external genitalia in 46,XY subjects. The total absence of adrenal androgens results in an adrenal crisis at birth. The same clinical presentation can be observed in deficiency of the side-chain cleavage enzyme (CYP11A). Very few cases of lipoid adrenal insufficiency have been reported in the medical literature.

A deficiency of 3β-HDS2 results in an inability of the gonads and adrenals to metabolize the Δ5 compounds (pregnenolone, 17-hydroxy pregnenolone, dehydroepiandrosterone) into Δ4 compounds (progesterone, 17-hydroxyprogesterone, androsten dione). Usually, one does not observe a complete 3β-HDS2 deficiency due to the fact that the body has other genes that can carry out some of the function of the 3β-HDS2 gene outside the gonads and adrenals. As a result, female fetuses are slightly overmasculinized, and male fetuses are markedly under-masculinized, at birth. In both sexes, salt wasting can occur as a result of inadequate production of cortisol and aldosterone. As previously noted, this form of enzyme deficiency occurs in females, and the result can be mild masculinization in adulthood. Such a situation is overlooked in males.

The 17-hydroxylase/17,20 desmolase enzyme both adds an OH group at position C-17 of the steroid molecule, and removes the side-chain. This, in turn, allows for the formation of 19-carbon (androgens) and 18-carbon (oestrogens) steroids. The 17-hydroxylase deficiency results in the accumulation of progesterone and pregnenolone with deficient cortisol, aldosterone, androgen and oestrogen production. A complete enzymatic deficiency results in a major adrenal crisis at birth, and female genitalia in a 46,XY subject. In 17,20 desmolase deficiency only, androgen and oestrogen secretion are compromised. Genetic males affected by 17,20 desmolase deficiency will thus present with female-appearing or ambiguous genitalia but no adrenal abnormality.

The enzyme 17β-hydroxysteroid dehydrogenase transforms the ketone of androstenedione into the 17β-hydroxyl group of testosterone. This enzyme is also needed to convert oestrone into oestradiol. Deficiency of 17β-hydroxysteroid dehydrogenase in 46,XY subjects results in the accumulation of androstenedione but no formation of testosterone. As androstenedione does not bind with androgen receptors (AR), there is an absence of androgenic effects during fetal development. Thus, a complete absence of 17β-hydroxysteroid dehydrogenase function leads to a 46,XY newborn with female genitalia. Most cases, however, consist of a partial enzyme abnormality, and these individuals present with ambiguous genitalia at birth if their
chromosome complement is 46,XY. At puberty, marked masculinization occurs in patients who have not had their testes removed, despite their inability to masculinize during fetal life. This may be explained by the presence of several 3β-hydroxysteroid dehydrogenase genes in the genome that are more active at puberty than during fetal life.

5α-Reductase deficiency

The 5α-steroid-reductase-2 enzyme metabolizes testosterone into DHT, a more potent androgen than testosterone. Deficiency of the 5α-steroid-reductase-2 enzyme results in under-masculinization of the genitalia in genetic males during fetal development. However, development of the Wolffian ducts proceeds normally. In addition, DHT is not necessary for the maturation of spermatocytes. Therefore, individuals with 5α-reductase deficiency can produce normal sperm in adulthood. Pubertal virilization (i.e. growth of the phallus, increased muscle mass and deepening of the voice) can also occur in these patients.

Androgen insensitivity syndrome (AIS)

A total insensitivity to androgen action is referred to as complete androgen insensitivity syndrome (CAIS). CAIS results from a mutation of the AR gene that is located on the long arm of the X chromosome near the centromere. Affected individuals exhibit female genitalia with normal testes located in the abdomen or inguinal area. As their testes produce normal amounts of MIS, testosterone and DHT in the context of end-organ insensitivity to androgens, both the Wolffian and Müllerian ducts fail to develop in affected subjects.

A diagnosis of CAIS is often made during surgery for bilateral hernia when testes are identified in the hernial sac of a girl or woman. In other cases, a diagnosis is made at puberty in young ladies who present with high concentrations of testosterone and DHT, amenorrhoea and no signs of virilization (particularly the absence of pubic and axillary hair).

Some mutations of the AR gene result in partial androgen insensitivity syndrome (PAIS). PAIS is characterized at birth by a 46,XY karyotype, ambiguous external genitalia, normal levels of testosterone and DHT, and elevated concentrations of LH. At puberty, the secondary sex characteristics of patients reared as males develop poorly and the phallus fails to grow. Female-typical breasts often develop in patients reared as males or females.

Practice points

- Identification of AR mutations is the gold standard for diagnosing AIS because other conditions, such as 17-ketosteroid reductase deficiency, can closely mimic the clinical presentation of AIS.
- Many of the women with AIS who participated in our long-range outcome study suffered from osteoporosis due to non-compliance with oestrogen-replacement therapy following the removal of their gonads. There is a need for education pertaining to the importance of oestrogen replacement for these women.
**Isolated persistence of Müllerian ducts**

This is an unusual condition in males that is detected at the time of hernial repair, when remnants of a uterus and fallopian tubes are observed in the hernial sac. In affected patients, testicular function is usually normal and the external genitalia are appropriately masculinized. It is probable that persistence of the Müllerian ducts is due to a mutation of MIS or the MIS receptors. It is also possible that Müllerian ducts exist in many males, in whom their presence is not usually detected.

**46,XY True hermaphroditism**

True hermaphroditism in a genetic male presents similarly to true hermaphroditism in a 46,XX individual. Both seminiferous tubules and ovarian follicles are present, resulting often in ovo–testis formation. The degree of masculinization of the genitalia and Wolffian ducts is directly related to the extent of testicular development of Leydig cells. The degree of development of the Müllerian ducts is related to the level of function of the Sertoli cells.

**Abnormal sex differentiation in patients with an abnormal sex chromosome complement**

Patients who possess an unusual sex chromosome complement can present with female external genitalia, ambiguous external genitalia or male external genitalia (Table 3). We will focus on those individuals who present with a female or an ambiguous phenotype only, as those who present with a male phenotype are not reared as girls and thus do not come to the attention of gynaecologists.

**Female external genitalia**

**Turner syndrome**

Girls and women who are affected by conditions of abnormal ovarian maintenance, referred to as Turner syndrome, present with normal female development except that they experience an unusually rapid fetal attrition of their germ cells resulting in streak gonads at birth. Approximately half of these patients have a 45,XO sex chromosome complement, and the remaining patients have a variant of this sex chromosome complement.

<table>
<thead>
<tr>
<th>External genital phenotype</th>
<th>Sex chromosome complement</th>
</tr>
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<tbody>
<tr>
<td>Female</td>
<td>45,X0 (Turner syndrome) and variants</td>
</tr>
<tr>
<td></td>
<td>47,XXX (super female)</td>
</tr>
<tr>
<td></td>
<td>46,Xip- or 46,Xi(Ys)</td>
</tr>
<tr>
<td>Ambiguous</td>
<td>45,XO/46,XY</td>
</tr>
<tr>
<td></td>
<td>46,XX/46,XY</td>
</tr>
<tr>
<td></td>
<td>Triploidy 69,XXY/69,YYY</td>
</tr>
<tr>
<td>Male</td>
<td>47,XXX (Klinefelter syndrome)</td>
</tr>
<tr>
<td></td>
<td>47,XXY</td>
</tr>
<tr>
<td></td>
<td>46,XX males</td>
</tr>
</tbody>
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complement (i.e. 46,XX with variable deletions of the long arm or the short arm of an X chromosome; 46,XX with an isochromosome for Xq). The presence of a small ring X chromosome results in a severe Turner condition that includes mental retardation. All types of Turner syndrome occur in approximately one in 5000 live births. The aetiology of X monosomy in patients can be a result of paternal or maternal meiotic nondysjunction. In 45,X0 girls and women, the remaining X is maternal in origin in 80% of cases.19 In Turner syndrome that is not 45,X0, the abnormal X chromosome is equally likely to be maternal or paternal in origin.

Along with gonadal streaks, most girls and women with Turner syndrome exhibit growth failure20 and any number of additional malformations that are associated with their condition. These malformations include cutis laxa or pterygium colli, lymphoedema, a low posterior hairline, micrognathia, ptosis of the upper eyelid, a shield chest with widely spaced nipples, a propensity for developing keloids and nevi, and horseshoe or unilateral kidneys. There can also be skeletal abnormalities and coartation of the aorta, as well as poor spatial processing skills in girls and women affected by Turner syndrome.4

The 47,XXX syndrome
Although this condition has been referred to as 'super female', a 47,XXX chromosome complement is associated with poor ovarian function and early menopause. These patients develop normal female genitalia.

The 46,XYp- or 46,Xi(Yq) chromosome complement
The presence of the SRY gene located at the tip of the short arm of the Y chromosome is necessary for the differentiation of a bipotential gonad into a testis. The deletion of SRY results in a syndrome of complete gonadal dysgenesis, with female external genitalia and Müllerian duct development coupled with the disappearance of the Wolffian ducts.

Ambiguous external genitalia
A 45,XO/46,XY karyotype
This condition is also termed mixed gonadal dysgenesis. This is because the gonads of these patients include one streak gonad (as in 45,XO Turner syndrome) and one poorly developed testis. Possibly, cloning of 45,XO cells in one gonad results in a streak while cloning of 46,XY cells results in the development of a testis. The degree of ambiguity of the external genitalia relates to the extent of testicular development, and thus testicular hormone production, in patients. Another aspect of mixed gonadal dysgenesis is short stature, similar to the condition of Turner syndrome.

Individuals with a male phenotype and normal fertility can possess a 45,XO/46,XY sex chromosome complement. Therefore, it seems that the development of individuals with a 45,XO/46,XY karyotype depends on the dominance of either 45,XO or 46,XY cell lines. The full spectrum of phenotypes associated with this atypical sex chromosome complement can thus be described as normal male genitalia at one end, female external genitalia at the other end, and mixed gonadal dysgenesis with ambiguous external genitalia in the middle.

A 46,XX/46,XY karyotype
This karyotype is rare and is thought to represent a chimera. Affected patients present with ambiguous genitalia, the degree of which is related to the relative dominance of 46,XX or 46,XY cells.
**Triploidy 69,XXY or 69,XYY**

This chromosome complement is rare and is usually observed in aborted fetuses. Only a few affected pregnancies result in a live birth, and for most of these, demise is rapid. It is of interest that affected newborns present with ambiguous genitalia.

**DETERMINATION OF THE AETIOLOGY OF AMBIGUOUS GENITALIA**

It is important to attempt to determine the aetiology of genital ambiguity as this may influence the decision to raise the child as a male or female. The first step is to obtain a karyotype (Figure 1). If the result is 46,XX, then you are most probably dealing with a masculinized female infant with ovaries and Müllerian structures. An exception to this would be 46,XX true hermaphroditism. If the karyotype is 46,XY, then you are dealing with an under-masculinized male related to either partial inability to produce, or respond to, testicular androgens.

In all cases of intersex conditions, the pattern of steroid hormone production must be established (i.e. testosterone, DHT, androstenedione, 17-hydroxyprogesterone and 17-hydroxypregnenolone) (Figure 2). This pattern can be studied in the basal condition before 3–4 months of age, or later in childhood following HCG stimulation. Low levels of testosterone coupled with elevated testosterone precursors will characterize a testosterone biosynthetic defect. If testosterone and its precursors are low, this can indicate partial gonadal dysgenesis, true hermaphroditism or Leydig cell hypoplasia. In contrast, normal values of testosterone with low levels of DHT suggest 5α-reductase deficiency. Finally, a normal testosterone/DHT ratio can indicate a potential diagnosis of PAIS. This diagnosis would need to be confirmed by detection of an androgen receptor gene mutation.

**Figure 1.** The first step—obtaining a karyotype—in determining the aetiology of ambiguous genitalia.
MANAGEMENT OF PATIENTS AFFECTED BY ABNORMAL SEX DIFFERENTIATION REARED AS FEMALES

The unique circumstances of every patient who presents with abnormal sex differentiation should be considered regardless of the underlying aetiology of their condition. Therefore, we recommend the following general guidelines, as opposed to a stringent set of rules, when treating affected patients.

A 46,XX chromosome complement

As indicated earlier, most cases of abnormal sex differentiation in individuals with a 46,XX chromosome complement are due to CAH. The major goals of medical management in these patients are to optimize growth and fertility with appropriate glucocorticoid and mineralocorticoid replacement, coupled with suppression of excess androgen production.\textsuperscript{12} Hormone replacement is based on a cortisol secretion rate of 5.7–10.0 mg/m\(^2\) body surface area/24 h. As cortisol is destroyed by gastric acid, the recommended daily oral replacement dose is about twice the normal secretion rate. Typically, a third of a dose is administered three times a day.

The only preparation available for aldosterone replacement is 9\(\alpha\)-fluoro-cortisol acetate (Florinef). Unlike cortisol replacement, aldosterone replacement is independent of body size. Doses of Florinef can range from 0.05 to 0.15 mg, taken orally once a day. Doses of Florinef that exceed 0.15 mg can produce hypertension.

As with all therapies, medical treatment of CAH must be adjusted for each patient. Routinely, plasma 17-hydroxyprogesterone and androstenedione concentrations are measured to assess the effectiveness of medical therapy. Generally, levels of 17-hydroxyprogesterone should be maintained between 500 and 1000 ng/dl, and levels of androstenedione should be maintained between 10 and 50 ng/dl. For control of
salt-water retention, plasma renin activity can be monitored. Finally, a bone-age measure every 12 months is useful.

Despite 50 years of experience with medical treatment for CAH, results using the previously mentioned therapies remain imperfect. Most patients do not reach a final height that is predicted by their genetic potential, and many are overweight. Adolescence is a particularly difficult time, with many patients becoming non-compliant with their medical therapy. For females, this non-compliance results in irregular menses and reduced fertility. Adrenalectomy has been proposed as a possible solution to poor suppression of excess adrenal androgens in affected girls and women. However, such an approach would be dangerous in patients already exhibiting non-compliance with their cortisol-replacement regimens.

Surgical treatment can include cosmetic procedures to feminize the appearance of the external genitalia when masculinization is marked in genetic females. Other surgical procedures may include vaginal construction. For some patients, vaginal dilation without surgical intervention is sufficient. There is currently a great deal of debate concerning the appropriate timing and type of genital surgical procedures.

The experience of our patients with vaginoplasty procedures performed in infancy is that, by adulthood, many required additional surgery to correct a small introitus. Several of our patients refused dilation or additional surgeries in adulthood. On this basis, we recommend that surgery to reduce the size of a large phallus be performed only in extreme cases in infancy, and that vaginal construction should wait until the patient is an adolescent or older.

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**Practice points**

- optimal medical treatment for children can be determined by monitoring growth rate and adrenal androgen suppression. Growth rate is best monitored with stadiometer and bone-age measurements
- CAH patients should be instructed on how to adjust their endocrine treatment in times of stress. Additionally, they should wear medic alert jewellery and be familiar with injectable preparations of cortisol replacement (i.e. Solu-Cortef). This is discussed at www.hopkinsmedicine.org/pediatricendocrinology/cah

**Research agenda**

- determine patient satisfaction with the various types and timing of genital surgical procedures
- determine the type and frequency of complications associated with each surgical procedure

Psychosexually, girls and women with CAH overwhelmingly report a female GI, along with some increased interest in male-typical play activities (GR). Debate over whether or not lesbianism occurs at increased rates in women with CAH remains unresolved.
Women who participate in our follow-up studies of 21-OH deficiency at Johns Hopkins frequently report that they are largely dissatisfied with their body image and also their lack of understanding of their medical condition. Some women are unhappy with their adult height (too short) and weight (too heavy). Other common body image complaints from this group include difficulties with conceiving and also problems with hirsutism. A number of our patients with 21-OH deficiency have expressed an interest in talking with other women who have similar medical histories to their own. Support groups can be useful to such patients as these groups can address two important areas in which patients with CAH are frequently dissatisfied. Members of support groups can act as an educational resource for others, and they can also provide the opportunity for meeting other people with common medical experiences. Two support groups that exist for people with CAH are The Magic Foundation (www.magicfoundation.org) and The Cares Foundation (www.caresfoundation.org).

A 46,XY chromosome complement

Female genitalia

46,XY subjects who present with female external genitalia related to any complete defect of masculinization (i.e. CAIS, Swyers syndrome, a complete testosterone biosynthetic defect) do not require cosmetic surgery to correct the appearance of their external genitalia. In all of these cases, a female sex of rearing is advised. We recommend removal of the gonads in these patients prior to puberty, preferably in the first year of life. This is because these patients are at risk for developing gonadoblastomas, and they may also masculinize at puberty if their diagnosis is not certain to be CAIS. Some patients will require vaginal lengthening; however, many will not.18 46,XY girls and women need oestrogen-replacement therapy to develop and maintain their female secondary sexual characteristics, to protect their bones, and also for menses in those who have a uterus (i.e. Swyers syndrome). It should be emphasized that patients with well-developed Müllerian ducts can undergo pregnancy with a donated fertilized egg, similar to women with Turner syndrome.

Ambiguous genitalia

The birth of a child with ambiguous genitalia is viewed as a major crisis by most parents. Paediatric endocrinologists, urologists, gynaecologists and psychologists with experience in this field can provide parents with information about normal sex differentiation and the specific abnormality that affects their child. The medical and surgical options should be explained along with our present knowledge of the long-range results of these treatments (i.e. the appearance of the sex organs, sexual function, reproduction, gender).23

In the most under-masculinized 46,XY infants, female sex of rearing is recommended. In newborns, a stretched phallus < 1.9 cm (−2.5 SDs) may not grow to a normal size, and perineo-scrotal hypospadias is difficult to correct along male lines. In more masculinized infants, male rearing can be successful. Patients reared as females will require oestrogen-replacement therapy. Except for those with a uterus, these women will be infertile. Similarly, patients raised as males will also require hormone therapy in the form of testosterone replacement, with the exception being those affected by 5α-reductase or 17β-hydroxysteroid dehydrogenase deficiency. Individuals with a diagnosis of 5α-reductase deficiency can be fertile if reared as males.
Our long-range outcome studies have shown that 46,XY patients with severe genital ambiguity, including perineo-scrotal hypospadias, required a greater number of genital surgeries when reared as males compared with those reared as females. Additionally, those raised as females had a better cosmetic outcome of their genital surgeries, as decided by physicians, than those reared as males. Somewhat surprisingly, subjects did not differ in their degree of satisfaction with their body image or sexual function. Among 26 patients reared as males, 76% were satisfied with their sex of rearing. Among 18 patients reared as females, 78% were satisfied with their sex of rearing. These results suggest that the development of gender identity is largely influenced by sex of rearing in the patients.

Similar to 21-OH deficiency, many of our 46,XY intersex patients have expressed an interest in talking with others who share similar medical histories. Our studies of these patients reveal that many desire a better understanding of their medical condition. Support groups that exist for these patients include the AIS Support Group (AISSG) at www.medhelp.org/www.ais. Multiple chapters of AISSG exist throughout the world.

45,XO chromosome complement

In the past 15 years, the medical treatment of girls with Turner syndrome has changed dramatically with the introduction of growth hormone to their therapy. The long-term effects of growth-hormone treatment are variable, and can range from adding 4–6 cm in some patients, to having very little benefit in others. In some cases, girls with Turner syndrome grow fast during pre-puberty, but then stop once oestrogen is administered. In most 45,XO girls, primary amenorrhoea occurs. In girls with a partial deletion of the long or short arm of one X chromosome, periods can occur for a variable amount of time. This is also the situation with 45,XO/46,XX mosaicism. Oestrogen replacement used to be initiated late in adolescence in order to permit optimal growth in affected girls. The use of growth hormone has allowed for earlier feminization with oestrogen treatment. Our practice has been to use small doses of oestrogen for 1 or 2 years, and then switch the patient to a contraceptive pill. Although most patients with Turner syndrome are infertile, pregnancies have occurred in these women. Support for girls and women with Turner syndrome can be viewed at www.turner-syndrome.org

SUMMARY

Abnormalities in human sex differentiation can present in a variety of ways, and can be attributed to a number of causes. We have attempted to review the basic steps of normal sex differentiation, followed by a consideration of the general patterns in which intersex conditions are observed. Additionally, we discuss steps for obtaining a differential diagnosis for the various intersex conditions that can occur. Included is a discussion on general management guidelines for girls and women affected by CAH, those affected by conditions of male pseudohermaphroditism, and those with Turner syndrome. Incorporated in these guidelines are medical and surgical treatment options, coupled with what is known about the long-term effects of these therapies. Finally, we have attempted to alert readers to the growing presence of patient support groups and the important role that these groups play in optimizing outcome for patients affected by syndromes of abnormal sex differentiation.
REFERENCES