Contents

Chapter 1
Ambiguous Genitalia

Meridith Pollie and Samantha M. Pfeifer

Case
A 3630-g infant was delivered vaginally 1 h ago after an uncomplicated 39-week gestation. On examination, the external genitalia are ambiguous. Vital signs are stable and the infant does not appear to be in acute distress. Examination shows small scrotal sacs resembling enlarged labia without palpable testes. An enlarged clitoris vs microphallus with hypospadias is seen. There is a small vaginal opening that appears to be partially fused. The remainder of the examination is normal.

Differential Diagnosis

The presenting symptom in this infant is ambiguous genitalia, or genitalia that do not appear typically male or female. The differential diagnosis of ambiguous genitalia in a newborn baby includes disorders of sexual development (DSD), in utero exposure to hormones that may masculinize female genitalia, insufficient androgen exposure in a male fetus, substances such as phenytoin and phenobarbital that may feminize male genitalia, and conditions of maternal hyperandrogenism such as maternal luteomas or theca lutein cysts that may also masculinize female genitalia. A comprehensive history should include hormone, drug, or substance exposure.

A disorder of sexual development is the most likely etiology in this infant. DSD are characterized by a mismatch between chromosomal/gonadal sex and genital

M. Pollie
Weill Medical College of Cornell University, New York, NY, USA

S. M. Pfeifer (✉)
Reproductive Medicine and Ob/Gyn, The Ronald Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Medical College of Cornell University, New York, NY, USA
e-mail: spfeifer@med.cornell.edu

P. H. Chung, Z. Rosenwaks (eds.), *Problem-Focused Reproductive Endocrinology and Infertility*, Contemporary Endocrinology,
https://doi.org/10.1007/978-3-031-19443-6_1

development, and etiologies include: genetic causes, referring to the presence or absence of functional sex chromosomes; signaling causes, referring to disrupted cellular communication during embryonic development; hormonal causes, referring to altered production or function of enzymes involved in hormone synthesis; and hormone receptor defects, which result in unsuccessful receptor-ligand interactions. Because developmental pathways are largely determined by an individual's karyotype, the latter is the cornerstone in guiding diagnosis of these patients.

For patients with XX karyotype, ambiguous genitalia result from pathologies involving high levels of androgens, either from the maternal environment or increased production in the fetal gonads, adrenal glands, or ectopic tissue. Congenital adrenal hyperplasia (CAH) includes a group of syndromes resulting from a deficiency in one of the enzymes needed to synthesize aldosterone and cortisol, leading to overproduction of androgens. More than 90% of CAH cases are caused by 21-hydroxylase deficiency, which leads to elevated serum levels of 17-hydroxyprogesterone (17-OHP) and androgens [1]. The most severe form of classic 21-hydroxylase deficiency can result in a salt-wasting syndrome due to dangerously low levels of serum aldosterone, leading to hyponatremia, hyperkalemia, metabolic acidosis, and inappropriate urine sodium excretion [1]. Infants with the salt-wasting syndrome are at risk for hypovolemic and hypoglycemic adrenal crises which can be fatal [1]. Other serum hormone levels, including cortisol, dehydroepiandrosterone, 17-hydroxypregnenolone, and 11-deoxycortisol, can help differentiate 21-hydroxylase deficiency from other, less common subtypes of CAH, including 3-beta-hydroxysteroid dehydrogenase deficiency or 11-beta-hydroxylase deficiency. Placental aromatase deficiency, an important diagnosis to consider in XX newborns, can lead to both ambiguous genitalia in the infant as well as maternal virilization due to excess serum testosterone which usually resolves after delivery.

Another important group of etiologies in XX individuals are mutations or translocations in genes involved in gonadal development. These include the translocation of the *SRY* gene, necessary in driving male gonadal development, from the Y-chromosome to the X-chromosome, mutations in the *SOX9* gene, which is a necessary transcription factor for testicular development, and mutations in *NR5A1*, which can lead to gain-of-function mutations causing inappropriate testicular tissue development (in XX individual) or loss-of-function mutations causing ineffective testicular differentiation (in XY individuals).

For patients with karyotype XY, syndromes that present with ambiguous genitalia are related to abnormally low levels or activity of the male sex hormones, testosterone, and dihydrotestosterone (DHT). Disorders of androgen synthesis include Smith-Lemli-Opitz syndrome (7-dehydrocholesterol reductase deficiency), P450 oxidoreductase deficiency, and 17,20 lyase deficiency. Contrastingly, 5-alpha-reductase deficiency is characterized by normal testosterone production but abnormal conversion of testosterone into DHT, impeding the DHT-dependent virilization of external genitalia. Androgen insensitivity syndrome (AIS), on the other hand, occurs when androgen hormone production is normal, but the androgen receptor is abnormal. This syndrome encompasses a spectrum of disorders from mild to partial or complete AIS that vary in presentation based on the degree of receptor

responsiveness to androgens and therefore the degree of virilization. Finally, patients with 46,XY/45,XO mosaicism may be diagnosed with Mixed Gonadal Dysgenesis (MGD) which entails a testis with Sertoli and Leydig cells but no germinal elements on one side and a streak gonad on the other. MGD is frequently due to mutations in the *WT1*, *SRY*, or *NR5A1* genes.

Evaluation

The first step in evaluating this patient with ambiguous genitalia is to screen for disorders that could be life-threatening, notably the salt-wasting form of CAH. Approximately 75% of patients with CAH have the salt-wasting type. Infants suffer from severe mineralocorticoid deficiency which can lead to Addisonian crisis and death if steroid supplementation is not initiated immediately [1]. Therefore, it is recommended that most infants with ambiguous genitalia should be screened immediately with serum electrolytes and 17-OHP levels. A karyotype can determine the child's chromosomal make-up and identify any mosaicism, but may take days to weeks to become available. Currently, many women undergo cell-free DNA testing in the first trimester to exclude Down Syndrome and may already know the fetal karyotype. These results should be confirmed postnatally. In our patient, initial screening labs revealed normal serum electrolytes and 17-OHP, and a prenatal test confirmed XY karyotype.

Once potentially fatal disorders are ruled out, a thorough history and physical exam should be the next steps in evaluation. History-taking should include specific questions about family history of consanguinity, infertility, or gonadal/urogenital malformations, maternal history of antenatal substance or medication use and any prior pregnancies, and maternal symptoms that may suggest androgen excess [2]. Initial examination of the infant should include evaluation of hydration status, jaundice, areolar hyperpigmentation, and blood pressure abnormalities, all of which may suggest altered levels of ACTH or mineralocorticoids. A focused genital exam should evaluate the presence or absence of testicular tissue, length of the clitoris/phallus, any fusion or rugosity of scrotal folds, hyperpigmentation, patency of the vaginal opening, and a digital rectal exam, which can reveal the presence of a uterus [2]. The external genitalia can be described using the Prader scale, which stages the degree of virilization using stages I (female with clitoromegaly) to V (male with hypospadias) [2]. Imaging with abdominopelvic ultrasound is helpful in determining the presence or absence of male/female internal genitalia. Our patient's family and maternal history was unremarkable, and examination revealed small scrotal sacs resembling enlarged labia without palpable testes, an enlarged clitoris vs microphallus with hypospadias, and a small vaginal opening that appears to be partially fused. The rectum was patent and no uterus was palpable on digital rectal exam. Abdominopelvic ultrasound revealed the absence of a uterus, upper vagina, fallopian tubes and ovaries, and the presence of bilateral intraabdominal testes.

Diagnostic workup should proceed with laboratory evaluation. In our patient, labs revealed elevated serum levels of testosterone, with normal levels of luteinizing hormone (LH), follicular stimulating hormone (FSH), and DHT. Elevated serum testosterone in a newborn is diagnostic of AIS and excludes the diagnosis of complete gonadal dysgenesis in which testosterone is not produced. Additional testing that is usually performed later in childhood includes human chorionic gonadotropin stimulation test to assess testosterone secretion by Leydig cells. Following the administration of human chorionic gonadotropin (hCG) (1000–2000 IU/day hCG for 3–5 days), an increase in testosterone (>200 ng/dL) suggests a diagnosis of AIS. Low serum testosterone after stimulation may suggest an alternative diagnosis impairing hormone synthesis, such as CAH. Normal testicular function can be further confirmed with serum anti-Mullerian hormone or inhibin, which should be within normal male limits in patients with AIS. Another useful test is to assess the ratio of serum testosterone to DHT, which should be normal in patients with AIS. An elevated testosterone/DHT ratio may suggest 5-alpha-reductase deficiency.

For patients with suspected AIS, genetic testing may offer the opportunity for definitive diagnosis. The androgen receptor (AR) gene is found on the long arm of the X chromosome and has eight exons that code for a protein of 919 amino acids [3]. Over 1000 genetic mutations causing AIS have been identified [3], initially using multiplex ligation-dependent probe amplification analysis, a type of polymerase chain reaction [4], and more recently, via next-generation and whole-exome sequencing [5]. AIS can be subclassified into complete, partial, and mild depending on the degree of responsiveness to androgens. Patients with complete AIS (CAIS) have the most severe form of androgen insensitivity and present with typical female external genitalia and testes with no internal female genitalia. CAIS has a global incidence estimated to range from 1 in 20,000 to 1 in 99,000 and is associated with identifiable mutations in more than 95% of cases [6, 7]. In CAIS patients, mutations have been identified in both exons and introns of AR gene, with the most common being missense mutations in the DNA-binding and the ligand-binding domains [3]. Mutations have also been found in CAIS patients in AR gene coactivators, helping to explain the phenotype in patients without mutations in the AR gene itself [8], as well as in the gene coding for 5-alpha-reductase [3], highlighting the overlapping features of these two disorders.

Partial AIS (PAIS) includes a spectrum of phenotypes characterized by varying degrees of masculinization of the external genitalia due to incomplete androgen responsiveness. The incidence of PAIS has been estimated to approach 1 in 130,000 [7]. Several limitations exist in genetic testing for patients with suspected PAIS. Firstly, while loss-of-function mutations in the AR gene can be found in nearly all CAIS patients, these mutations can be found in less than 50% of PAIS patients [9]. Some PAIS mutations have been found instead in the hinge region connecting DNA-binding domain and the ligand-binding domain [3]. In addition, there is no genotype-phenotype correlation in PAIS, which is to say that two individuals with the same gene mutation may have distinct clinical presentations [6]. Finally, some cases of PAIS may result from post-zygotic mutations leading to somatic mosaicism of normal wild-type receptors and abnormally mutated receptors, which

may lead to falsely normal genetic testing results [10]. In patients without identifiable mutations, androgen binding assays may provide an alternative way to quantify AR responsiveness and function.

In this section, based on karyotype, imaging, and laboratory evaluation, we have established the most likely diagnosis for our patient as PAIS.

Treatment

The next step in management of an infant determined to have PAIS should be to assemble a multidisciplinary team made up of pediatricians, endocrinologists, pediatric urologists, geneticists, and counselors who specialize in DSD and who can work together to guide the family with medical management and emotional support and guidance. Providers should acknowledge that no treatment exists to prevent or reverse abnormal development in embryogenesis in patients with PAIS. It should also be recognized that initial gender uncertainty can be distressing for families and language that pathologizes the condition should be avoided. Instead, differences in sexual development should be presented as anatomic variations. The words normal and abnormal should be replaced by typical and atypical. Case series estimate that 5–15% of PAIS children will experience gender dysphoria regardless of the sex they are raised with [11, 12]. These discussions should aim to communicate that, in infancy, it is impossible to determine the child's eventual gender identity. Therefore, parents can be encouraged to choose a gender-neutral name and prepare to rear the child with expectations that gender identity may change as the child ages. Current recommendations emphasize the importance of patient autonomy, age-appropriate education, and shared decision-making. This marks a drastic shift from previous practice of nondisclosure, an approach which stemmed from the medico-ethical climate of the 1950s that involved concealing the diagnosis of AIS from patients in an attempt to avoid confusion and minimize patient anxiety [13]. Today, ongoing psychological support for both the patient and family comprises an integral part of management and can include psychotherapy, support groups, and strong continuity with medical professionals [14].

An important question that arises in the management of patients with AIS is whether and when to pursue gonadectomy. For patients with CAIS, bilateral gonadectomy was historically recommended in childhood as cryptorchid testes were thought to carry increased risk for malignant testicular germ cell tumors [15]. However, recent literature suggests that rates of tumorigenesis in patients with CAIS may be as low as 2% [14]. Studies suggest that risk for malignant transformation may be slightly higher in patients with abdominal versus inguinal testes [16]. However, recent practice has shifted toward deferring gonadectomy until late adolescence in CAIS patients for several reasons. First, the maintenance of endogenous androgen secretion through puberty allows for bone mineral accumulation, breast development, and a growth spurt via peripheral aromatization of androgens to estrogens [15]. Second, recent evidence shows that testicular tumors do not develop until

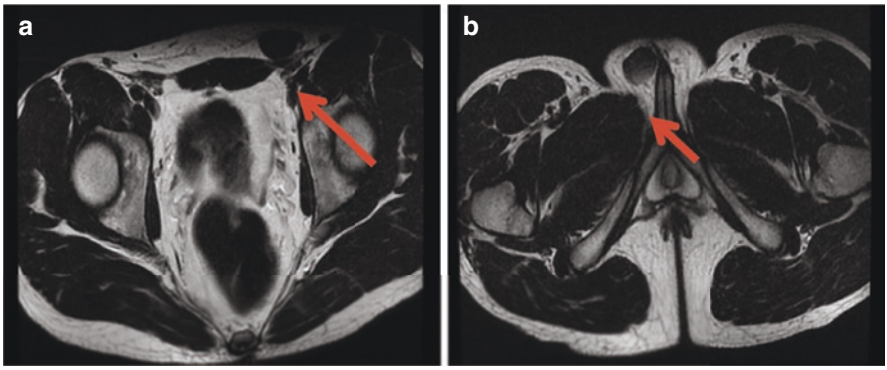

Fig. 1.1 Pelvic MRI of patient with PAIS. (**a**) Arrow showing left inguinal gonad. (**b**) Arrow showing right labial/scrotal gonad

after puberty [17, 18], with the earliest reported case of malignancy with CAIS in a 14-year-old [17]. Therefore, in order to uphold patient autonomy in decision-making, puberty can be deferred if needed with GnRH agonists until the patient can reach appropriate age to make an informed decision about gonadectomy. Gonadectomy can be performed sooner if the patient is experiencing labial or inguinal discomfort or if there is an indication for other abdominal surgery in order to prevent the need for multiple operations.

Contrastingly, risk for germ cell malignancy in patients with PAIS is estimated to approach 50% in patients with intra-abdominal testes and is unknown in patients with intrascrotal testes [14] (Fig. 1.1). As a result, for PAIS patients with intra-abdominal testes, current recommendations are for gonadectomy at time of diagnosis, while those with scrotal testes can avoid surgery and instead undergo testicular biopsy at puberty, though the data supporting this recommendation is limited [14]. If a PAIS patient's parents elect against early gonadectomy, bilateral orchiopexy should be performed at the time of diagnosis in order to facilitate examination and sonographic surveillance.

Some CAIS or PAIS patients may elect to forego surgery and retain their testes in spite of potential elevated cancer risk. These patients need monitoring with regular screening for testicular malignancy. Suggested surveillance protocols for these patients include baseline imaging with transabdominal ultrasound (US) and magnetic resonance imaging (MRI) to localize and characterize gonads, followed by annual transabdominal or pelvic ultrasounds and examinations depending on the location of the testes [7]. Baseline exam under anesthesia or diagnostic laparoscopy may also be considered in some patients, particularly those with equivocal imaging findings [7]. Other proposed screening regimens include biannual follow-up with both US and MRI along with serial tumor markers (hCG, alpha-fetoprotein, lactate dehydrogenase) and hormonal assessment (FSH, LH, testosterone, inhibin B) [19], though the additive value of tumor markers is controversial [7]. In patients with a new finding on annual screening imaging (i.e., calcifications, cysts, a mass,

lymphadenopathy, size change, or new asymmetry), MRI should be performed, followed by possible diagnostic laparoscopy for direct visualization with possible biopsy and/or gonadectomy [7].

Patients with AIS should also be counseled about their prospects for fertility. Infertility in male-identifying patients with AIS is almost universal [20], but assisted reproductive technologies may allow for success. In CAIS testes reveal incomplete spermatogenesis, increased fibrosis, Leydig cell hyperplasia, and low frequency of spermatogonia. In PAIS individuals some androgen receptor function is preserved but not usually enough to promote adequate sperm production. Individuals with mild AIS may be diagnosed due to presence of severe oligospermia at the time of an infertility evaluation. As gonadal germ cells have been shown to be present in CAIS patients, some have proposed that the same techniques currently in use for fertility preservation in pediatric cancer patients be utilized with these patients [21]. However, as it has been shown that the number of gonadal germ cells may inversely correlate with patient age, some propose removal and cryopreservation of testicular germ cells prior to age 2 [22]. However, these procedures and timing of such are still considered experimental. For individuals with PAIS who identify as male, therapy with clomiphene citrate [23] and high-dose testosterone therapy [24] have been proposed to improve semen parameters and fertility. In patients with known heritable mutations, risk for intergenerational transmission of the disease to female offspring via the X chromosome should be discussed, and preimplantation genetic screening can be offered [21]. For female-identifying patients with AIS who desire pregnancy, options include surrogacy or potentially uterus transplant in the future, as currently individuals with a 46 XY karyotype are not candidates for this experimental procedure.

Once a patient with AIS reaches puberty, additional interventions may be indicated depending on the patient's gender identity. For AIS individuals who identify a gender other than that assigned to them at birth or in childhood, hormone therapy or surgical treatment may help to alleviate distress and affirm their desired gender expression. Hormone replacement therapy (HRT) is indicated in all individuals post-gonadectomy either at the time of expected puberty or post-puberty, depending on the timing of surgery. For those who identify as female and had a gonadectomy prior to puberty, estrogen supplementation should be initiated to induce breast development, puberty, and augment bone density. Estrogen should be initiated at the lowest dosage and gradually increased over approximately 2 years to support physiologic breast development. Individuals should then be maintained on a dosage of estrogen appropriate for a young female, which is higher than that for postmenopausal females [6]. In individuals with PAIS who identify as male, high-dose testosterone supplementation is well-documented to have a positive impact with regard to virilization [25, 26]. In individuals with CAIS who identify as female, testosterone therapy has been shown to enhance sexual function without impacting adversely on psychological well-being [27]. Some individuals with CAIS are interested in adding testosterone therapy to estrogen replacement to see if they feel "better." It is important to tailor the hormone regimen to the individual and titrate the dose to optimize well-being, secondary sexual characteristics, and bone density.