Sex Determination and Differentiation

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Sex determination, which depends on the sex-chromosome complement of the embryo, is established by multiple molecular events that direct the development of germ cells, their migration to the urogenital ridge, and the formation of either a testis, in the presence of the Y chromosome (46,XY), or an ovary in the absence of the Y chromosome and the presence of a second X chromosome (46,XX). Sex determination sets the stage for sex differentiation, the sex-specific response of tissues to hormones produced by the gonads after they have differentiated in a male or female pattern. A number of genes have been discovered that contribute both early and late to the process of sex determination and differentiation. In many cases our knowledge has derived from studies of either spontaneous or engineered mouse mutations that cause phenotypes similar to those in humans. We will examine how mutations in these genes cause important clinical syndromes (Table 1 and Fig. 1) and discuss clinical entities that continue to elude classification at the molecular level. Knowledge of the molecular basis of disorders of sex determination and differentiation pathways will continue to have a strong influence on the diagnosis and management of these conditions. Terminology, when possible, adheres to that used in the Online Mammalian Inheritance in Man data base developed by the National Center for Biotechnology Information of the National Library of Medicine (http://www.ncbi.nlm.nih.gov).

**GERM CELLS**

Primordial germ cells, which eventually localize in the gonad, first appear in the proximal epiblast, the outer ectodermal layer of the embryo, whence they migrate through the primitive streak and then to the base of the allantois, where they can be identified by alkaline phosphatase staining. The germ cells then migrate along the wall of the hindgut to the urogenital ridge, the site of the future gonad (Fig. 2). Interesting factors that specify the fate of these primordial germ cells have recently been elucidated in mice. Two genes that are unique to the differentiating germ cells are Fragilis and Stella. Fragilis is first detected in the proximal epiblast, where its expression is influenced by the bone morphogenetic protein 4 (BMP4), then in the base of the allantois, where the expression of Stella commences. On migration of the germ cell to the genital ridge, the expression of Fragilis diminishes while that of Stella persists. Inactivation of BMP4 is associated with inhibition of the expression of Stella and Fragilis and results in the absence of germ cells, which attests to the necessity of these genes in germ-cell formation and development. However, it is not clear how either Fragilis or Stella specifies the fate of germ cells. Fragilis belongs to an interferon-inducible family of transmembrane proteins involved in transducing antiproliferative signals and in adhesion, both of which may be important in the coalescence of germ cells at the base of the allantois. Stella transcribes a novel protein, the structure of which suggests that it may have a role in RNA processing and chromatin modification. This protein is thought to maintain the pluripotent state of the migrating primordial germ cells by silencing transcription of genes specific to somatic cells. Only
The proliferation patterns of male and female germ cells differ. XY germ cells undergo mitosis during migration but soon after reaching the gonads, their growth becomes arrested and they remain within the testis in the quiescent (G₀) phase of the cell cycle until after birth under the influence of an unknown inhibitory factor (referred to as meiosis inhibitory factor) secreted by either Sertoli or myoid cells. After birth, the male germ cells resume the cell cycle and undergo meiotic division, which halves the number of chromosomes to produce haploid spermatogonia. The Sertoli cells nurture the germ cells, which complete spermatogenesis at puberty under the influence of the gonadotropins follicle-stimulating hormone and luteinizing hormone from the pituitary. Important to this process are proteins secreted by Sertoli cells, including cytokines, müllerian inhibiting substance, inhibin, activin, and insulin-like growth factor I.

**MALE GERM CELLS**

XX germ cells undergo mitosis as they migrate to the female genital ridge and enter the ovary; the cells then progress through the initial stages of the first meiotic division, becoming arrested at prophase 1 by birth (Fig. 2). At this stage the surviving germ cells become surrounded by a single layer of somatic granulosa cells, and in mice, a stimulatory adenylcyclase maintains the oocyte in this primordial follicular state. Communication between oocytes and the surrounding granulosa cells occurs when the resting primordial follicles are stimulated to grow at the time of puberty as primary, secondary, and preovulatory follicles under the influence of follicle-stimulating hormone. Also, oocyte-derived growth and differentiation factor 9 and BMP15, along with zona pellucida proteins 1, 2, and 3, act synergistically with granulosa-cell products, surprisingly similar to those secreted by Sertoli cells, to maintain the oocyte and to control ovulation.

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Table 1. Mutations in Genes Involved in Sex Determination and Development and Associated with Intersex Anomalies.

<table>
<thead>
<tr>
<th>Gene (Locus)</th>
<th>Protein and Proposed Function</th>
<th>Mutant Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT1 (11p13)</td>
<td>Transcription factor</td>
<td>Frasier syndrome, Denys–Drash syndrome with Wilms’ tumor</td>
</tr>
<tr>
<td>SF-1 (9q33)</td>
<td>Transcription factor, nuclear receptor</td>
<td>Gonadal and adrenal dysgenesis</td>
</tr>
<tr>
<td>SOX9 (17q24)</td>
<td>High-mobility-group transcription factor</td>
<td>Campomelic dysplasia, male gonadal dysgenesis or XY sex reversal</td>
</tr>
<tr>
<td>DAX1 (Xp21.3)</td>
<td>Transcriptional regulator, nuclear-receptor protein</td>
<td>Gonadal dysgenesis, congenital adrenal hypoplasia</td>
</tr>
<tr>
<td>SRY (Yp11)</td>
<td>High-mobility-group transcription factor</td>
<td>Gonadal dysgenesis</td>
</tr>
<tr>
<td>MIS, or AMH, type II receptor (12q12–13)</td>
<td>Serine threonine kinase receptor</td>
<td>Persistent müllerian duct syndrome</td>
</tr>
<tr>
<td>MIS, or AMH (19p13)</td>
<td>Secreted protein, causes regression of fetal müllerian duct; Leydig-cell inhibitor</td>
<td>Persistent müllerian duct syndrome</td>
</tr>
<tr>
<td>AR (Xq11–12)</td>
<td>Androgen receptor, a ligand transcription factor</td>
<td>Male pseudohermaphroditism, complete or partial androgen insensitivity syndrome</td>
</tr>
<tr>
<td>HSD17B3 (9q22)</td>
<td>17β-Hydroxysteroid dehydrogenase, 17-ketosteroid reductase 3</td>
<td>Male pseudohermaphroditism</td>
</tr>
<tr>
<td>SRD5A2 (5p15)</td>
<td>5α-Reductase type 2</td>
<td>Male pseudohermaphroditism*</td>
</tr>
<tr>
<td>CYP17 (10q24–25)</td>
<td>17-Hydroxylase: 20–22 lyase</td>
<td>Male pseudohermaphroditism</td>
</tr>
<tr>
<td>CYP21 (6q21.3)</td>
<td>21-Hydroxylase</td>
<td>Congenital adrenal hyperplasia, female pseudohermaphroditism</td>
</tr>
<tr>
<td>HSD3B2 (1p13.1)</td>
<td>3β-Hydroxysteroid dehydrogenase type II</td>
<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>CYP11B1 (8q24)</td>
<td>11β-Hydroxylase</td>
<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>STAR (8p11.2)</td>
<td>Steroidogenic acute regulatory protein</td>
<td>Congenital lipoid adrenal hyperplasia</td>
</tr>
</tbody>
</table>

* Virilization may occur at puberty.
Germ cells are absent in the mutant strain of piebald mice and in “Sertoli-only” testes of infertile men who have deletions in the long arm of the Y chromosome in the azoospermia factor (AZF) regions that control spermatogenesis. The recent elucidation of the sequence of the human Y chromosome will provide a template to further our understanding of the structure and function of this chromosome, particularly of the elusive long arm (q). Stem-cell factor, a ligand also known as mast-cell growth factor that is encoded by the steel locus on chromosome 12q, acts through its receptor, c-kit, and is important for the migration and survival of germ cells. Stem-cell factor, basic fibroblast growth factor, and the glycoprotein 130 (gp 130) ligands lymphocyte inhibiting factor and interleukin-6 are all essential in immortalizing germ cells in vitro. These specialized germ cells, in turn, can form embryoid bodies, which when injected into blastocysts can colonize all cell lineages.

The isolation of embryonic germ cells led to the development of immortalized germ cells and eventually to the immortalization of human and primate embryonic pluripotent stem cells derived either

Figure 1. Clinical Examples of Intersex Abnormalities.
Panel A shows the internal structures of a 46,XY patient with pure gonadal dysgenesis. There are bilateral streak gonads with retained müllerian structures, fallopian tubes, and a midline uterus. Panel B shows severe clitoral hypertrophy caused by masculinization of the external genitalia of a 46,XX patient with female pseudohermaphroditism caused by congenital adrenal hyperplasia. Panel C shows incomplete masculinization of the external genitalia of a 46,XY patient with male pseudohermaphroditism. There is a microphallus with perineoscrotal hypospadias and bifid and prepenile scrotum. Panel D shows internal genitalia of a 46,XY patient with persistent müllerian duct syndrome. There are müllerian structures (i.e., fallopian tubes and uterus) as well as wolffian structures (i.e., the vas deferens and epididymis). Panel B is reprinted from Donahoe and Crawford with the permission of the publisher.
from fetal specimens or from excess blastocysts generated by in vitro fertilization protocols. These developments have increased our understanding of factors affecting pluripotency and have fueled hopes that therapeutic cloning can be used to create differentiated cell types for replacement therapy. Thus, germ-cell biology has contributed to the development of stem-cell biology. In turn, discoveries regarding pluripotency have also led to myriad ethical controversies and initiated steps to ensure that cells will not be unlawfully for reproductive cloning of humans.

**Figure 2. Migration and Proliferation of Germ Cells during Embryonic and Fetal Life.**

Germ cells are first detected in the epiblast, where they are activated by bone morphogenetic protein 4 (BMP4) from the extraembryonic ectoderm. Migration occurs through the primitive streak to the base of the allantois, where alkaline phosphatase–positive cells can be detected. Subsequently, the cells migrate to the urogenital ridge, where the gonads form. The 46,XY and 46,XX germ cells undergo different patterns of mitosis and meiosis.

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**Syndromes of Gonadal Dysgenesis**

Investigations of the molecular events that occur during sex determination, coupled with an analysis of the phenotypes of mice in which candidate genes have been inactivated by homologous recombination (knockout mice), have increased our understanding of the pathophysiology of some of the clinical defects that are characterized by gonadal dysgenesis. As germ cells are migrating, the urogenital ridge forms from the intermediate mesoderm under the influence of a number of factors, including the transcription factors empty-spericles homeobox gene 2 (Emx2), GATA-4, Lim1, and Lim homeobox 9 (Lhx9) (Fig. 3). Mutations in the genes for these factors produce abnormal gonads in mice, but similar mutations have not yet been implicated in gonadal-dysgenesis syndromes in humans. However, three genes encode interacting proteins that are critical for the formation of the urogenital ridge in humans. The products of the Wilms' tumor-suppressor gene (WT1) are essential for both gonadal and renal formation. The steroidogenic factor 1 (SF-1) and the duplicated in adrenal hypoplasia congenita on the X chromosome (DAX 1) proteins are essential for gonadal and adrenal differentiation (Fig. 3). Our discussion of the clinical gonadal-dysgenesis syndromes will illustrate the important roles that these molecules play in the pathogenesis of the disorders.
The Frasier Syndrome and WT1

The Frasier syndrome is characterized by both gonadal dysgenesis and renal abnormalities that result in streak gonads coupled with the nephrotic syndrome (Fig. 3). If it occurs in the XY genotype then there is sex reversal. Study of the phenotype of Wt1− knockout mice revealed that the gene is involved in the early steps of the differentiation of both gonads and kidneys, helping to explain the association of gonad and kidney malfunction in the Frasier syndrome.

Alternative splicing of the Wt1 gene in mice can result in up to 24 protein isoforms. Mutations of two of these isoforms lead to striking clinical manifestations, thereby demonstrating their importance in human sex determination. They are the −KTS and the +KTS variants, in which there is deletion (−) or maintenance (+), respectively, of three amino acids, lysine (K), threonine (T), and serine (S) between the third and fourth zinc fingers of the DNA-binding domain of this transcription factor. Hammes et al. found that altering the expression of KTS in mice influences both kidney and testicular function. In the Frasier syndrome, the splice site of Wt1 that normally preserves the KTS triplet is mutated; therefore, patients with the syndrome produce only WT1 protein without KTS. Gonads lacking KTS have decreased production of the sex-determining region of the Y chromosome (SRY), a urogenital ridge protein that is critical for testicular differentiation. In these −KTS gonads there is also a decrease in müllerian inhibiting substance, a glycoprotein hormone derived from Sertoli cells that causes regression of the male müllerian ducts and whose presence is an early marker of testicular differentiation. The findings
in the Frasier syndrome indicate that the +KTS WT1 isoform must be produced either at the same time or before the urogenital ridge produces the SRY that will induce gonadal differentiation. Persons with a 46,XY karyotype will have a female phenotype with retained müllerian ducts as well as nephropathy. The severity of the nephropathy varies, however, with the position of the mutation that disrupts the KTS region; some genotypes lead to renal failure in infancy, whereas others cause milder forms of nephrotic syndrome compatible with increased longevity. Patients with the Frasier syndrome who have a mutation that inactivates KTS, however, are not susceptible to Wilms’ tumor.

The Denys–Drash Syndrome and WT1
Mutations outside the KTS region result in a WT1 protein that affects gonads later in development, leading to the Denys–Drash syndrome, in which gonads differentiate more completely than the gonads of patients with the Frasier syndrome. Thus, affected patients have a less severe functional deficiency. For example, male gonads are sufficiently developed to produce müllerian inhibiting substance, which ensures that regression of the müllerian ducts is normal, but the synthesis of testosterone is impaired. Although persons with a 46,XY karyotype have a predominantly male phenotype, low testosterone levels can cause male pseudohermaphroditism with various degrees of hypospadias and undescended testes. Patients with the Denys–Drash syndrome also have a high incidence of Wilms’ tumors and a nephropathy characterized by focal glomerular and mesangial sclerosis, which often results in end-stage renal disease and ultimately renal transplantation in the second or third decade of life.

These multiple molecular WT1 variants resulting from alternative splicing of the KTS amino acid triplet have different clinical implications. Study of patients with the ~KTS mutation has alerted clinicians to the fact that phenotypic girls with focal glomerular sclerosis or the nephrotic syndrome should be screened for XY sex reversal. Also, phenotypic girls with XY sex reversal who retain müllerian structures because the gonadal dysgenesis occurs before the production of müllerian inhibiting substance should be screened for the nephrotic syndrome. In addition, boys with mild undervirilization characterized by hypospadias and undescended testes who also have proteinuria may have the Denys–Drash (+KTS) variant and should be monitored carefully for focal glomerular nephropathy and Wilms’ tumor.

Wilms’ tumor can be associated with aniridia, genitourinary anomalies, and mental retardation—the WAGR syndrome. These complex phenotypic associations are thought to occur because of the proximity of WT1 on chromosome 11p13 to the paired box homeotic (PAX6) gene and two other genes in that region that are expressed in the embryonic brain. Patients with the Beckwith–Weidemann syndrome of hemihypertrophy, caused by mutations of a gene on chromosome 11p15, are also prone to Wilms’ tumor.

GONADAL AND ADRENAL ABNORMALITIES
Steroidogenic Factor
Another important gene in early gonadal development is SF-1, which encodes a transcription factor homologous to steroid hormone receptors, but whose ligand is unknown, placing the receptor in a class of orphan nuclear hormone receptors. SF-1 binds DNA and regulates the expression of a number of genes that participate in sexual development. These include müllerian inhibiting substance and all the cytochrome P-450 steroid hydroxylase enzymes and 3β-hydroxysteroid dehydrogenase, which are required for the synthesis of sex steroid hormones. SF-1–knockout mice fail to develop adrenal glands and gonads and die at birth. A human with adrenal insufficiency and 46,XY sex reversal was found to have a mutation in SF-1, W1 and SF-1 have been shown to interact in mice, with Wt1 enhancing the effect of SF-1 on downstream genes.

DAX1
The DAX1 gene codes for a member of the nuclear-receptor family of proteins. Since this protein lacks a DNA-binding domain but does have a ligand-binding domain, it presumably regulates gene expression through protein–protein interaction. DAX1 mutations are associated with adrenal hypoplasia congenita, a syndrome of adrenal insufficiency due to impaired development of the adrenal cortex, and hypogonadotropic hypogonadism as a result of impaired development of the pituitary and the gonads (Fig. 3). Dax1 antagonizes the synergy between SF-1 and Wt1 in mice, thereby inhibiting the transcription of SF-1 downstream genes, most likely by recruiting corepressors or by blocking binding of SF-1 to DNA.
A Potential Role for DAX1 in Mixed Gonadal Dysgenesis

An intersex disorder resulting in dysgenetic and often asymmetric gonads is the enigmatic syndrome of mixed gonadal dysgenesis, which is most often associated with a mosaic 45,X/46,XY karyotype, although a 46,XY karyotype is found in 40 percent of patients. The mosaicism is characterized by the presence of at least two gonadal germ-cell lines with different chromosomal complements. The percentage of cells with an intact XY genotype dictates the degree of testicular differentiation. In the classic form, there is a streak gonad on one side and a dysgenetic fibrotic testis with disordered tubular architecture on the other, retained müllerian ducts caused by a deficiency of müllerian inhibiting substance, and incomplete genital masculinization as a result of a deficiency of testosterone. It is not clear why gonadal asymmetry is such a prominent feature of mixed gonadal dysgenesis, but it is probably related to sex-chromosome mosaicism. The streak gonad, resembling those seen in patients with Turner’s syndrome, is thought to result from a loss of the Y chromosome owing to embryonic nondysjunction, the failure of paired chromosomes to migrate to opposite poles during mitosis or meiosis. The phenotype of patients with mixed gonadal dysgenesis can vary, with gonads that are more normal at birth than those in patients with pure gonadal dysgenesis (see below) but that undergo early degeneration, which may progress to dysgenesis and subsequent neoplastic transformation. Because of the possibility of neoplastic transformation, early removal of the gonads is recommended. Less severe phenotypes can occur if the same 46,X/46,XY karyotype is found in a relatively small percentage of chimeric cells. In fact, 45,X/46,XY karyotypes are now being found incidentally in phenotypically normal males owing to the increased frequency of prenatal genetic testing.

An X-linked molecule like DAX1 may have a role in mixed gonadal dysgenesis (Fig. 3 and 4), since DAX1 suppresses testicular differentiation. The presence of two X chromosomes, albeit in different cells, one from 45,X0 and the other from 46,XY, may be sufficient to prevent sustained testicular growth and differentiation by providing excessive DAX1 (or another inhibiting molecule), which suppresses testicular development. This concept is supported by the observation that the presence of a second X chromosome in XXY humans with Klinefelter’s syndrome and in XXY mice leads to abnormalities of germ-cell development with early entry into meiosis.

SRY and SRY Homeobox Genes in Pure Gonadal Dysgenesis

Patients with pure gonadal dysgenesis have bilateral streak gonads that fail to differentiate. Analysis of these patients and animal models led to the discovery of the SRY gene located on the distal short arm of the Y chromosome and to the detection on autosomes of SRY homologues, such as the SRY homeobox gene SOX9. The molecular basis for testicular differentiation became more clear when phenotypic males were produced after an Sry transgene was introduced into XX mice, confirming the role of Sry as a genetic switch that induces testicular differentiation. Mutations in the DNA-binding region of the SRY gene, which is a member of a large high-mobility-group family, were found in a subgroup of 46,XY sex-reversed females with pure gonadal dysgenesis. These patients have characteristic bilateral streak gonads, which are small and fibrotic, without the typical germ-cell or supporting-cell morphology of testes or ovaries. Campomelic dysplasia, a severe disorder characterized by 46,XY sex reversal, streak gonads, and severe skeletal malformation, occurs in patients with a translocation in the distal arm of chromosome 9p near the SRY-related SOX9 gene and other genes associated with sex reversal in lower organisms. SOX9 and SRY are co-expressed in the male but not the female urogenital ridge, implicating the two genes in testis determination (Fig. 3). The fact that SOX9 activates the transcription of müllerian inhibiting substance further supports the idea that it has a crucial role in male gonadal development.
The gonads have less severe dysgenesis than do the gonads of patients with mixed gonadal dysgenesis. The molecular events leading to this unique disorder have not been elucidated, but a few cases have been attributed to translocation of a fragment containing the SRY gene to a cryptic site on the X chromosome.

**MÜLLERIAN AGENESIS**

The undifferentiated gonad coexists with both male and female reproductive ducts. The paramesonephric, or müllerian, duct forms the uterus, fallopian tubes, and the upper vagina, and under the influence of testosterone, the mesonephric, or Wolffian, duct forms the vas deferens, seminal vesicles,
and epididymides. A transcription factor gene common to the development of both müllerian and wolffian systems is PAX2. This gene is required for normal intermediate development of the mesoderm in both sexes; mutations in mice lead to müllerian-duct, wolffian-duct, and renal agenesis. A mutation in the PAX2 gene has been reported in a family with the renal-coloboma syndrome, which partially reproduces the results seen in mice. Failure of müllerian development occurs in 46,XX female patients with Mayer–Rokitansky–Küster–Hauser syndrome, which is characterized by vaginal or complete müllerian agenesis and kidney abnormalities, including a pelvic kidney or the more severe agenesis of the kidney. Inactivation of Wnt-4, the gene encoding a member of the Wingless family of proteins, may be implicated in this disorder. Wnt is an acronym for a drosophila homologue of the Wingless family of proteins that is found in the mouse genome at a site where the mouse mammary tumor virus growth factor often integrates. The Wnt-4 protein is secreted by the müllerian-duct epithelium and induces the development of the müllerian mesenchyme. Early inactivation of Wnt-4 causes failure of the formation of müllerian-duct derivatives in both sexes; however, a functional effect is manifested only in females, since in normal males, the müllerian duct regresses under the influence of müllerian inhibiting substance. Coincident kidney defects are lethal at birth in mice, but humans with less severe phenotypes can survive.

Homeobox (Hox) transcription factors 9, 10, 11, and 13 are necessary for normal uterine and vaginal development; abnormalities in the expression of the genes for these factors account for some uterine and vaginal atresias. Mutations in a Hox13 allele are the cause of the hand–foot–genital syndrome, in which there are deformities of the hands and feet, vaginal abnormalities in females or hypospadias in males, spinal abnormalities, and extrophy of the bladder and cloaca. Transfection of constructs with this mutation into the chicken-hindgut region reproduced these abnormalities. Diethylstilbestrol has been known since 1971 to alter müllerian development. The fact that diethylstilbestrol suppresses another Wnt gene — Wnt-7a — and alters Hox gene expression in müllerian ducts in mice provides a plausible molecular mechanism for the uterine abnormalities, vaginal adenosis, and rarely, carcinoma observed in patients who were exposed to diethylstilbestrol in utero.

FUNCTIONAL ABNORMALITIES IN SEXUAL DEVELOPMENT

After normal morphologic development of the gonads, loss-of-function mutations of testicular proteins such as müllerian inhibiting substance or the müllerian inhibiting substance receptor can lead to the development of disorders characterized by retained müllerian ducts. Failure to produce testosterone or mutations in the testosterone receptor can produce 46,XY phenotypic females or phenotypic males with various degrees of diminished masculinization. Conversely, patients with congenital adrenal hyperplasia produce an excess of adrenal androgens, which can cause female pseudohermaphroditism in 46,XX patients.

PERSISTENT MÜLLERIAN DUCT SYNDROME

Persistent müllerian duct syndrome occurs in 46,XY males as a rare form of male pseudohermaphroditism that is caused by a defect in either the gene for the müllerian inhibiting substance, located on chromosome 19p13, or its type II receptor, located on chromosome 12q13 (Fig. 4). Patients with this syndrome have retained müllerian ducts and unilateral or bilateral undescended testes, and they may also have crossed testicular ectopia caused by herniated uterine structures, which drag the contralateral gonad into one scrotum with its ipsilateral gonad.

MALE PSEUDOHERMAPHRODITISM

Another important cause of male pseudohermaphroditism with sexual ambiguity is failure of androgen production or an inadequate response to androgen, both of which can cause incomplete masculinization of persons with the 46,XY karyotype. The clinical spectrum varies from mild failure of masculinization, with hypospadias and undescended testes, to complete sex reversal (Fig. 4) with a female phenotype. Androgen-receptor mutations result in the androgen insensitivity syndrome in which testes can be intraabdominal or in the inguinal canals, but wolffian structures and external genitalia fail to respond to high levels of testosterone and its target-tissue metabolite dihydrotestosterone. Adequate müllerian inhibiting substance produced by the otherwise normal testes, however, results in complete regression of müllerian ducts.

Another cause of undervirilization arises from
defects in the synthesis of testosterone in patients with mutations in the steroidogenic enzymes responsible for the conversion of cholesterol to dihydrotestosterone — namely, steroidogenic acute regulatory protein, cytochrome P-450 17-hydroxylase, 3β-hydroxysteroid dehydrogenase, and 17-ketosteroid reductase. These defects cause low levels of androgen. Mutations in the 5α-reductase type 2 gene result in low levels of dihydrotestosterone, which cause penoscrotal hypospadias, prepenile scrotum, and an enlarged prostatic utricle, often requiring surgical reconstruction. As in the androgen insensitivity syndrome, regression of the müllerian duct occurs because the normal Sertoli cells produce normal or even elevated levels of müllerian inhibiting substance. Many genetic males with a deficiency of 5α-reductase type 2 are born with female external genitalia and are raised as females. The curious virilization that occurs in these patients at puberty often leads to a change in sexual identity. This paradox is explained by a normal increase at puberty in the activity of the 5α-reductase type 1 isoform, which results in sufficient dihydrotestosterone to complete the virilization of these genetic males.

### CONGENITAL ADRENAL HYPERPLASIA

Congenital adrenal hyperplasia is caused by the inability of the adrenal to synthesize sufficient cortisol, leading to excess testosterone and resulting in severe masculinization in 46,XX females. More severe forms involve decreased aldosterone production and salt wasting. The most common mutation occurs in the cytochrome P-450 21-hydroxylase enzyme; a less common form (5 percent of cases) results from a loss-of-function mutation in 3β-hydroxysteroid dehydrogenase. Rarer still is 11β-hydroxylase deficiency, which can also result in prenatal or postnatal virilization. Insufficient production of cortisol and the resultant failure of negative feedback in the hypothalamic–pituitary–adrenal axis causes excess corticotropin production, leading to adrenocortical hyperplasia. In addition, cortisol precursors are shuttled to other steroid pathways, causing high levels of adrenal androgenic steroids, which masculinize the female external genitalia to form a glans penis, rather than a clitoris, and scrotum, rather than labia majora (Fig. 4). Under the influence of the excess androgens, the vagina fails to complete its descent to the perineum, causing a common urogenital canal or sinus with incomplete separation of the vagina and urethra. Ovaries and müllerian structures are otherwise normal, because their development is independent of sex steroids at this stage. The diagnosis can be made in utero, and early maternal dexamethasone therapy can ameliorate the masculinized phenotypes. Surgical reconstruction can be performed in infancy to restore the female phenotype.

### SUMMARY

The study of patients with syndromes characterized by ambiguous genitalia and associated anomalies, together with analyses of spontaneous and engineered mutations causing similar abnormalities in animals, has elucidated many of the molecular defects causing sex reversal and disorders of reproductive function in humans. Our knowledge is expanding regarding the molecular events necessary to initiate the development of the urogenital ridge and to select and sustain further sex differentiation and development of gonads, reproductive ducts, and external genitalia. This deeper understanding has, in some cases, contributed to improved patient care both by increasing the likelihood of a positive outcome and by averting unfavorable events. This knowledge must be incorporated into treatment strategies in order to increase and sustain the function, happiness, and emotional fulfillment of patients with abnormalities of sex differentiation.

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