

REVIEW ARTICLE

MECHANISMS OF DISEASE

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>).

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N Engl J Med 2004;350:367-78.

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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.² 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.² 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.³ Only

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

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

* Virilization may occur at puberty.

the germ cells that reach the presumptive gonadal region differentiate and survive; germ cells outside this region undergo apoptosis, although some escape and can later become germ-cell tumors.⁴

MALE GERM CELLS

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 cells⁵ (Fig. 2). 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.⁶

FEMALE 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.⁷ 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.⁸ Also, oocyte-derived growth and differentiation factor 9 and BMP15, along with zona pellucida proteins 1, 2, and 3,⁹ act synergistically with granulosa-cell products, surprisingly similar to those secreted by Sertoli cells, to maintain the oocyte and to control ovulation.

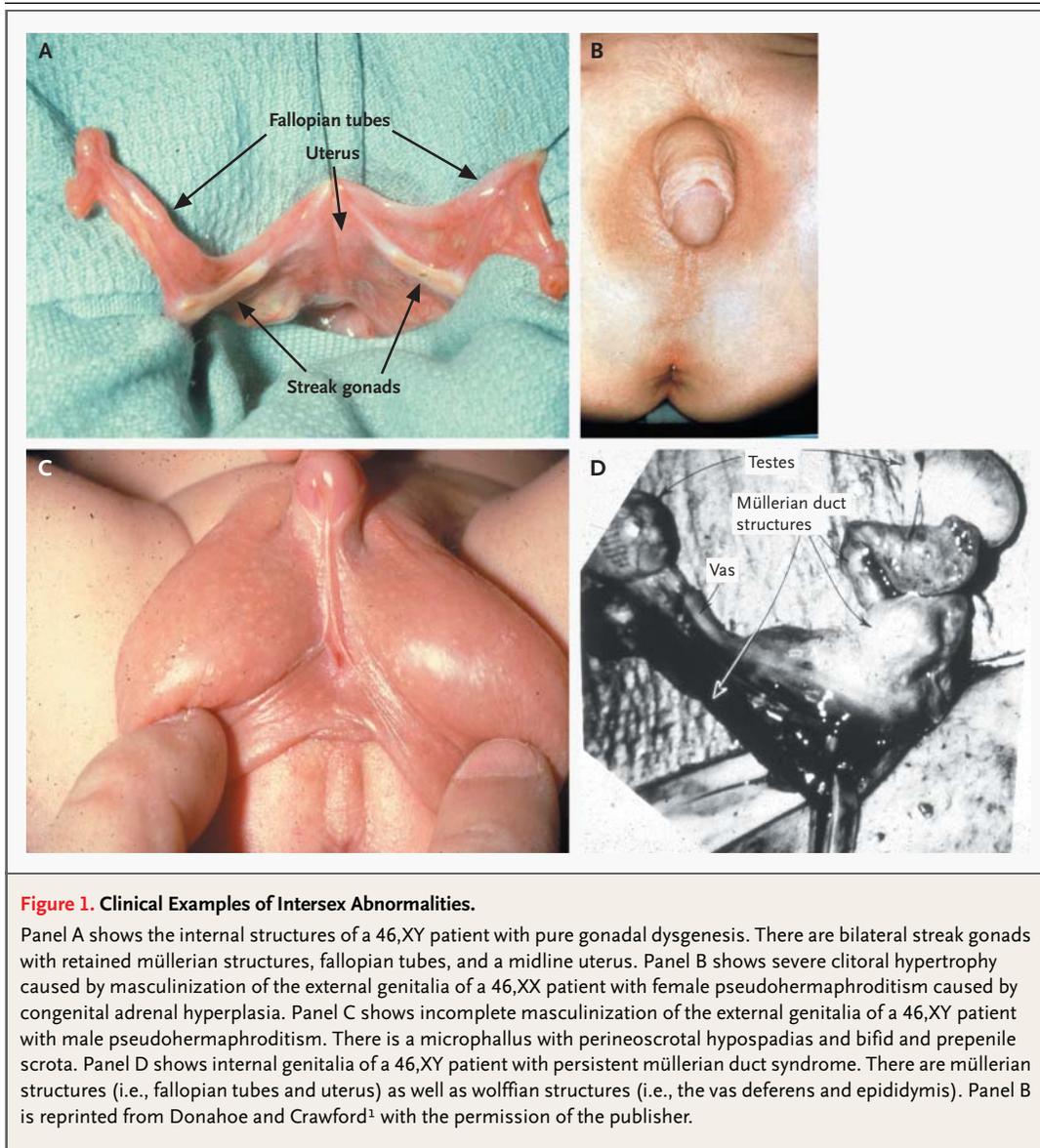


Figure 1. Clinical Examples of Intersex Abnormalities.

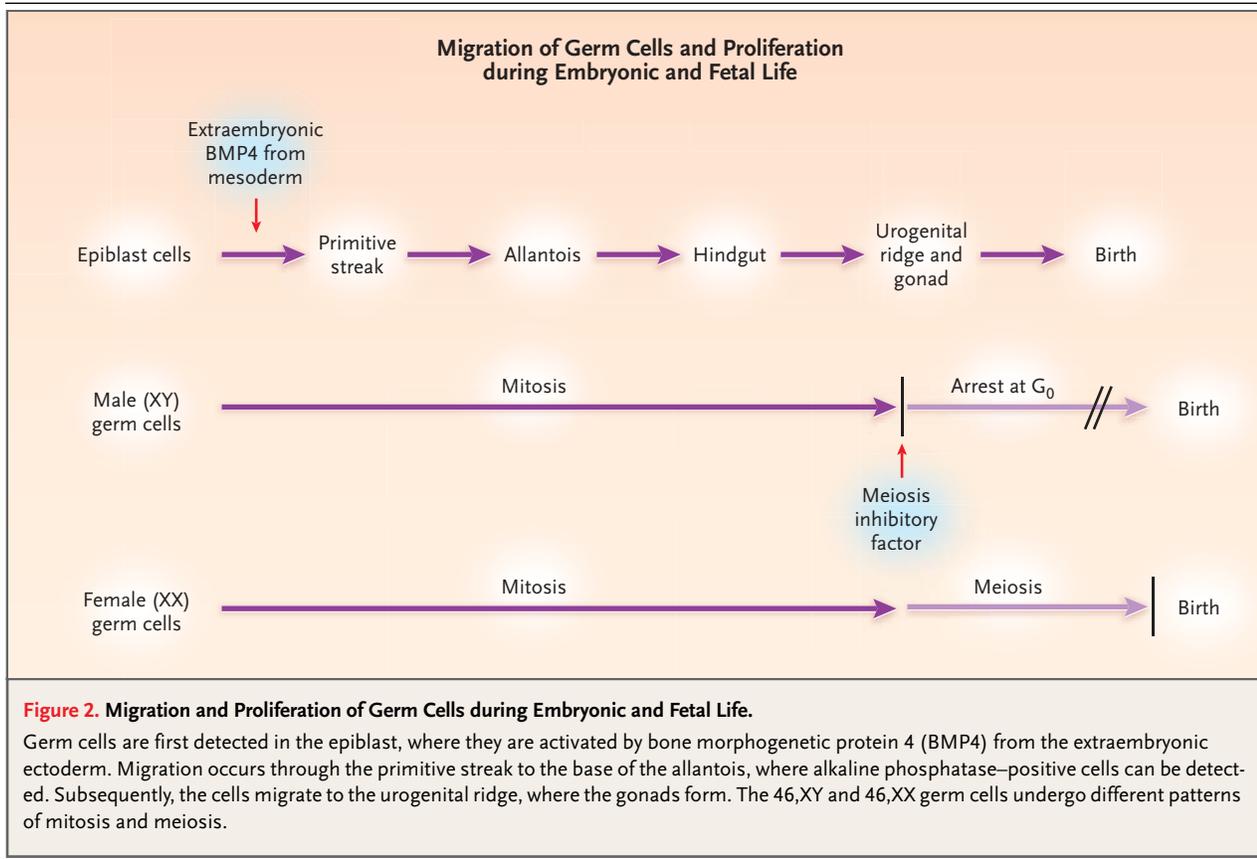
Panel A shows the internal structures of a 46,XY patient with pure gonadal dysgenesis. There are bilateral streak gonads with retained müllerian structures, fallopian tubes, and a midline uterus. Panel B shows severe clitoral hypertrophy caused by masculinization of the external genitalia of a 46,XX 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 scrota. 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 Crawford¹ with the permission of the publisher.

SYNDROMES OF ABSENT GERM CELLS AND RELATION OF GERM CELLS TO STEM CELLS

Germ cells are absent in the mutant strain of piebald mice¹⁰ and in “Sertoli-only”¹¹ testes of infertile men who have deletions in the long arm of the Y chromosome in the azoospermia factor (AZF) regions that control spermatogenesis.¹² The recent elucidation of the sequence of the human Y chromosome¹³ 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,¹⁴ 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,¹⁵ and the glycoprotein 130 (gp 130) ligands lymphocyte inhibiting factor and interleukin-6 are all essential in immortalizing germ cells in vitro.¹⁵ These specialized germ cells, in turn, can form embryoid bodies, which when injected into blastocysts can colonize all cell lineages.¹⁶

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



from fetal specimens¹⁷ or from excess blastocysts generated by in vitro fertilization protocols.¹⁸ 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 controversies¹⁹ and initiated steps to ensure that cells will not be used unlawfully for reproductive cloning of humans.

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 gene 2 (*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.

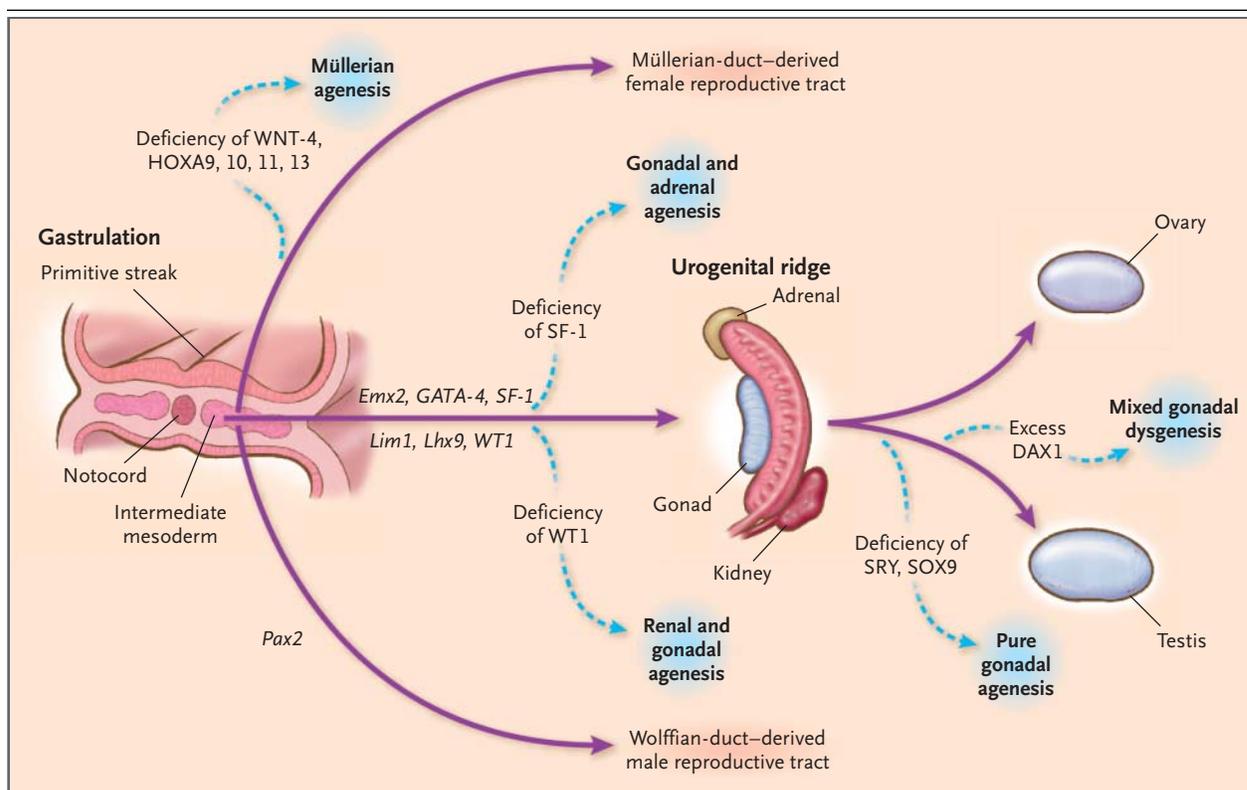


Figure 3. Syndromes of Dysgenesis during the Development of the Urogenital Ridge.

Mutations in various genes can lead to a variety of syndromes of dysgenesis involving the müllerian or wolffian ducts, gonads, kidneys, and adrenal glands as a result of a deficiency or excess of the proteins shown. *DAX1* denotes the gene for duplicated in adrenal hypoplasia congenita on the X chromosome 1; *Emx2* the empty spericles homeobox gene 2; *GATA-4* the gene encoding a protein that binds to a GATA DNA sequence; *HOXA* homeobox protein; *Lim1* a homeobox gene important for limb development; *Lhx9* a lim homeobox family member; *PAX2* paired box homeotic gene; *SF-1* the gene for steroidogenic factor 1; *SRY* sex-determining region of the Y chromosome; *SOX9* SRY homeobox 9; *Wnt-4* a protein that induces the development of the müllerian mesenchyme; and *WT1* Wilms' tumor-suppressor gene 1.

GONADAL AND RENAL ABNORMALITIES

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.²⁰ 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.²¹ 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.²² 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.²³ 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,²⁴ 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-1,²⁵ 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^{21,26-28} and all the cytochrome P-450 steroid hydroxylase enzymes²⁹ and 3 β -hydroxysteroid dehydrogenase,³⁰ 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.³¹ A human with adrenal insufficiency and 46,XY sex reversal was found to have a mutation in SF-1.^{32,33} Wt1 and Sf-1 have been shown to interact in mice, with Wt1 enhancing the effect of Sf-1 on downstream genes.³⁴

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.³⁵ DAX1 mutations are associated with adrenal hypoplasia congenita,³⁶ 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 Wt1³⁴ in mice, thereby inhibiting the transcription of Sf-1 downstream genes, most likely by recruiting corepressors³⁷ or by blocking binding of Sf-1 to DNA.³⁸

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,³⁹ although a 46,XY karyotype is found in 40 percent of patients.⁴⁰ The mosaicism is characterized by the presence of at least two gonadal germ-cell lines with different chromosomal complements.⁴¹ 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,³⁹ which may progress to dysgenesis and subsequent neoplastic transformation. Because of the possibility of neoplastic transformation, early removal of the gonads is recommended.^{39,40} 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.⁴¹

An X-linked molecule like DAX1 may have a role in mixed gonadal dysgenesis (Fig. 3 and 4), since DAX1 suppresses testicular differentiation.³⁵ The presence of two X chromosomes, albeit in different cells, one from 45,XO 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.⁴²

SRY AND SRY HOMEBOX 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.⁴³ 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.⁴⁴ 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 gene⁴⁵ and other genes associated with sex reversal in lower organisms.⁴⁶ 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^{28,47} further supports the idea that it has a crucial role in male gonadal development.

TRUE HERMAPHRODITISM

An unusual cause of ambiguous genitalia is true hermaphroditism; in this syndrome, both ovarian and testicular tissue is present either in the same or in a contralateral gonad. This disorder is rare in North and South America but quite common in Africa and the Middle East. Asymmetry of gonads and subsequently of reproductive ducts and external genitalia is common, with testes, ovaries, and ovotestes present in various combinations in patients with a predominantly 46,XX karyotype.⁴⁸ The sex of rearing is dictated by the phenotype, which is directed by the predominant gonad. In true hermaphroditism,

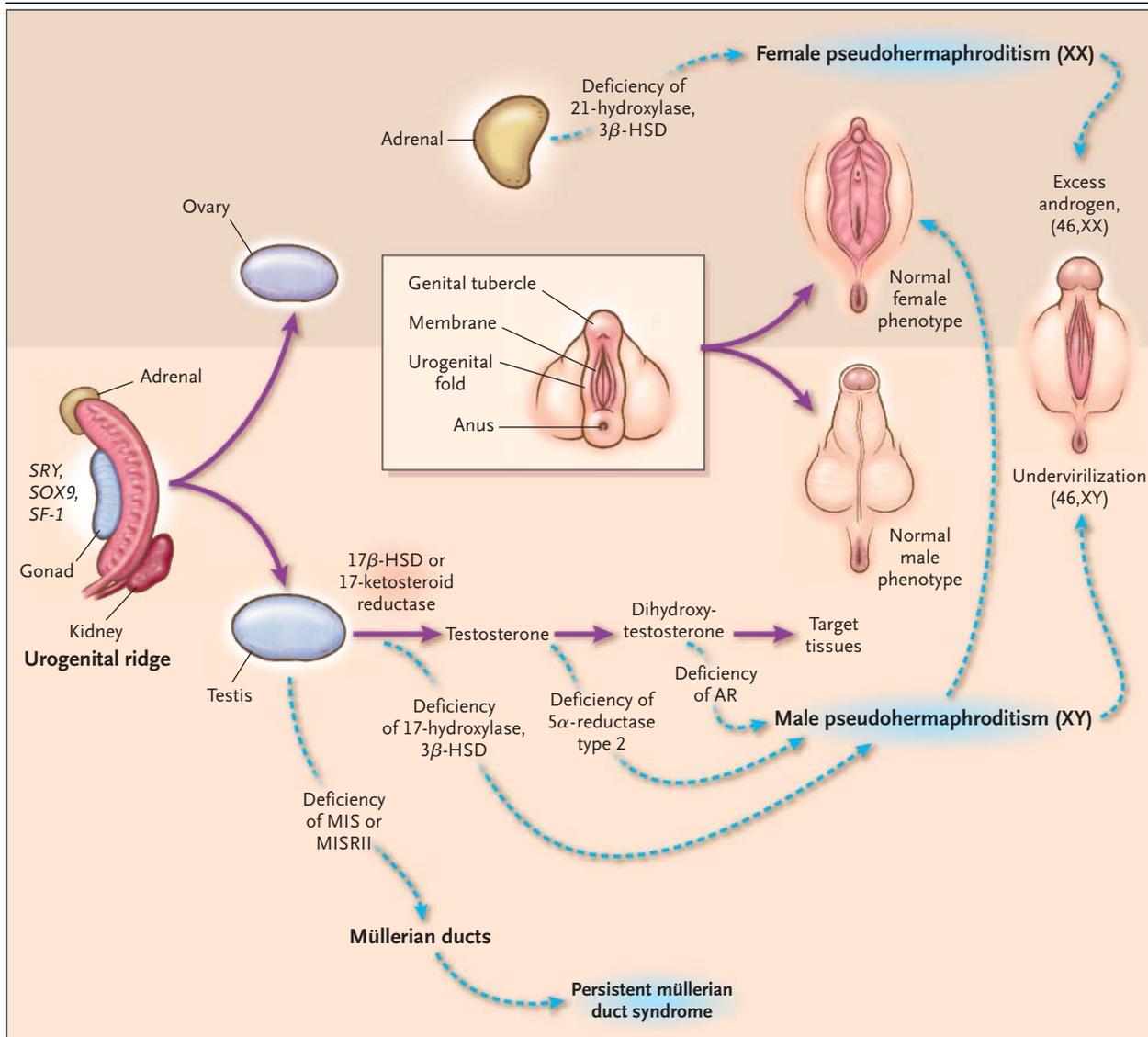


Figure 4. Functional Abnormalities of the Synthesis and Action of Hormones.

After the gonads have formed, reduced hormonal activity or signaling of specific receptors can lead to functional abnormalities of the reproductive tract, including persistent müllerian duct syndrome; male pseudohermaphroditism, causing undervirilization; and müllerian agenesis. After adrenal development, reduced enzymatic activity can result in female pseudohermaphroditism with excessive virilization. HSD denotes hydroxysteroid dehydrogenase, MIS müllerian inhibiting substance, MISRII müllerian inhibiting substance type II receptor, *SF-1* the gene for steroidogenic factor 1, *SRY* the gene for the sex-determining region of the Y chromosome, *SOX9* the gene for *SRY* homeobox 9, and AR androgen receptors.

the gonads have less severe dysgenesis⁴⁹ 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.⁵⁰

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 wolfian, 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.⁵¹ A mutation in the *PAX2* gene has been reported in a family with the renal-coloboma syndrome,⁵² 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.⁵³ 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,^{54,55} 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.⁵⁶ Mutations in a *Hoxa13* 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.⁵⁷ Transfection of constructs with this mutation into the chicken-hindgut region reproduced these abnormalities.⁵⁸ Diethylstilbestrol has been known since 1971⁵⁹ to alter müllerian development. The fact that diethylstilbestrol suppresses another *Wnt* gene — *Wnt-7a* — and alters *Hox* gene expression in müllerian ducts in mice⁶⁰ provides a plausible molecular mechanism for the uterine abnormalities, vaginal adenosis, and rarely, carcinoma observed in patients who were exposed to diethylstilbestrol in utero.⁵⁹

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,^{21,61–63} located on chromosome 19p13,⁶⁴ or its type II receptor, located on chromosome 12q13⁶⁵ (Fig. 4). Patients with this syndrome⁶¹ 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 scrotum⁶⁶ 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^{67,68} 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,⁶⁹ cytochrome P-450 17-hydroxylase,⁷⁰ 3 β -hydroxysteroid dehydrogenase,⁷¹ and 17-ketosteroid reductase.⁷² These defects cause low levels of androgen. Mutations in the 5 α -reductase type 2 gene⁷³ result in low levels of dihydrotestosterone, which cause penoscrotal hypospadias, prepenile scrota, and an enlarged prostatic utricle,^{73,74} often requiring surgical reconstruction.⁷⁵ As in the androgen insensitivity syndrome, regression of the müllerian duct occurs because the normal Sertoli cells produce normal or even elevated⁷⁶⁻⁷⁸ levels of müllerian inhibiting substance. Many genetic males with a deficiency of 5 α -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.⁷⁹ This paradox is explained by a normal increase at puberty in the activity of the 5 α -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 synthesize⁸⁰ 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.⁸⁰ The most common mutation occurs in the cytochrome P-450 21-hydroxylase enzyme⁸¹⁻⁸³; a less common form (5 percent of cases) results from a loss-of-function mutation in 3 β -hydroxysteroid dehydrogenase.⁷¹ Rarer still is 11 β -hydroxylase deficiency, which can also result in prenatal or postnatal virilization.^{84,85} 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 androgen-

ic steroids, which masculinize the female external genitalia to form a glans penis, rather than a clitoris, and scrota, 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.^{80,83,86} Surgical reconstruction can be performed in infancy to restore the female phenotype.⁸⁷

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.

Supported in part by grants from National Institute of Child Health and Human Development (HD 32112) and the National Cancer Institute (CA 17393).

We are indebted to Alex Arango, Ph.D., Liz Perkins, Ph.D., and Christopher Houk, M.D., for insightful comments and suggestions; to John D. Crawford, M.D., Pediatric Endocrinology, and his colleagues, with whom we have worked collaboratively over the past 30 years; to the Department of Surgery and the Pediatric Surgical Services, which have sustained the clinical care of these patients and the basic research that has led to improved understanding of the pathophysiology underlying their disorders.

REFERENCES

1. Donahoe PK, Crawford JD. Ambiguous genitalia in the newborn. In: Welch KJ, Randolph JG, Ravitch MM, O'Neill JA Jr, Rowe MI, eds. *Pediatric surgery*. 4th ed. Vol. 2. Chicago: Year Book Medical, 1986:1363-82.
2. Saitou M, Barton SC, Surani MA. A molecular programme for the specification of germ cell fate in mice. *Nature* 2002;418:293-300.
3. Matova N, Cooley L. Comparative aspects of animal oogenesis. *Dev Biol* 2001;231:291-320.
4. Talerman A. Germ cell tumours. *Ann Pathol* 1985;5:145-57.
5. McLaren A, Buehr M. Development of mouse germ cells in cultures of fetal gonads. *Cell Differ Dev* 1990;31:185-95.
6. Matsumoto A. *Spermatogenesis*. Philadelphia: Lippincott-Raven, 1996:359-84.
7. Mehlmann LM, Jones TL, Jaffe LA. Meiotic arrest in the mouse follicle maintained

- by a Gs protein in the oocyte. *Science* 2002; 297:1343-5.
8. Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. *Science* 2002;296:2178-80.
 9. Prasad SV, Skinner SM, Carino C, Wang N, Cartwright J, Dunbar BS. Structure and function of the proteins of the mammalian Zona pellucida. *Cells Tissues Organs* 2000; 166:148-64.
 10. Evans EP, Breckon G, Peters J. An XXY mouse, the result of a rearrangement between one X and a Y chromosome. *Genet Res* 1990;56:15-9.
 11. Ma K, Sharkey A, Kirsch S, et al. Towards the molecular localisation of the AZF locus: mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. *Hum Mol Genet* 1992;1:29-33.
 12. Kuroda-Kawaguchi T, Skaletsky H, Brown LG, et al. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. *Nat Genet* 2001;29:279-86.
 13. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 2003;423: 825-37.
 14. Dolci S, Williams DE, Ernst MK, et al. Requirement for mast cell growth factor for primordial germ cell survival in culture. *Nature* 1991;352:809-11.
 15. Koshimizu U, Taga T, Watanabe M, et al. Functional requirement of gp130-mediated signaling for growth and survival of mouse primordial germ cells in vitro and derivation of embryonic germ (EG) cells. *Development* 1996;122:1235-42.
 16. Labosky PA, Barlow DP, Hogan BL. Mouse embryonic germ (EG) cell lines: transmission through the germline and differences in the methylation imprint of insulin-like growth factor 2 receptor (Igf2r) gene compared with embryonic stem (ES) cell lines. *Development* 1994;120:3197-204.
 17. Shambloot MJ, Axelman J, Wang S, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci U S A* 1998;95:13726-31. [Erratum, *Proc Natl Acad Sci U S A* 1999;96: 1162.]
 18. McLaren A. Mammalian germ cells: birth, sex, and immortality. *Cell Struct Funct* 2001;26:119-22.
 19. Executive summary. In: Scientific and medical aspects of human reproductive cloning. Washington, D.C.: National Academy Press, 2002.
 20. Hammes A, Guo JK, Lutsch G, et al. Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. *Cell* 2001; 106:319-29.
 21. Teixeira J, Maheswaran S, Donahoe PK. Mullerian inhibiting substance: an instructive developmental hormone with diagnostic and possible therapeutic applications. *Endocr Rev* 2001;22:657-74.
 22. Little M, Holmes G, Bickmore W, van Heyningen V, Hastie N, Wainwright B. DNA binding capacity of the WT1 protein is abolished by Denys-Drash syndrome WT1 point mutations. *Hum Mol Genet* 1995;4:351-8.
 23. Miller RW, Fraumeni JF Jr, Manning MD. Association of Wilms's tumor with aniridia, hemihypertrophy and other congenital malformations. *N Engl J Med* 1964;270:922-7.
 24. Koufos A, Grundy P, Morgan K, et al. Familial Wiedemann-Beckwith syndrome and a second Wilms tumor locus both map to 11p15.5. *Am J Hum Genet* 1989;44:711-9.
 25. Parker KL, Schedl A, Schimmer BP. Gene interactions in gonadal development. *Annu Rev Physiol* 1999;61:417-33.
 26. Shen WH, Moore CC, Ikeda Y, Parker KL, Ingraham HA. Nuclear receptor steroidogenic factor 1 regulates the Mullerian inhibiting substance gene: a link to the sex determination cascade. *Cell* 1994;77:651-61.
 27. Arango NA, Lovell-Badge R, Behringer RR. Targeted mutagenesis of the endogenous mouse MIS gene promoter: in vivo definition of genetic pathways of vertebrate sexual development. *Cell* 1999;99:409-19.
 28. Watanabe K, Clarke TR, Lane AH, Wang X, Donahoe PK. Endogenous expression of Mullerian inhibiting substance in early postnatal rat Sertoli cells requires multiple steroidogenic factor-1 and GATA-4-binding sites. *Proc Natl Acad Sci U S A* 2000; 97:1624-9.
 29. Parker KL, Schimmer BP. Transcriptional regulation of the genes encoding the cytochrome P-450 steroid hydroxylases. *Vitam Horm* 1995;51:339-70.
 30. Leers-Sucheta S, Morohashi K, Mason JJ, Melner MH. Synergistic activation of the human type II 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase promoter by the transcription factor steroidogenic factor-1/adrenal 4-binding protein and phorbol ester. *J Biol Chem* 1997;272:7960-7.
 31. Lala DS, Rice DA, Parker KL. Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. *Mol Endocrinol* 1992;6:1249-58.
 32. Achermann JC, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. *Nat Genet* 1999;22:125-6.
 33. Taketo M, Parker KL, Howard TA, et al. Homologs of *Drosophila* fushi-tarazu factor 1 map to mouse chromosome 2 and human chromosome 9q33. *Genomics* 1995; 25:565-7.
 34. Nachtigal MW, Hirokawa Y, Enyeart-VanHouten DL, Flanagan JN, Hammer GD, Ingraham HA. Wilms' tumor 1 and Dax-1 modulate the orphan nuclear receptor SF-1 in sex-specific gene expression. *Cell* 1998; 93:445-54.
 35. Parker KL, Schimmer BP, Schedl A. Genes essential for early events in gonadal development. *Cell Mol Life Sci* 1999;55: 831-8.
 36. Ito M, Yu R, Jameson JL. DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita. *Mol Cell Biol* 1997; 17:1476-83.
 37. Crawford PA, Dorn C, Sadovsky Y, Milbrandt J. Nuclear receptor DAX-1 recruits nuclear receptor corepressor N-CoR to steroidogenic factor 1. *Mol Cell Biol* 1998;18: 2949-56.
 38. Zazopoulos E, Lalli E, Stocco DM, Sassone-Corsi P. DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. *Nature* 1997;390:311-5.
 39. Donahoe PK, Crawford JD, Hendren WH. Mixed gonadal dysgenesis, pathogenesis, and management. *J Pediatr Surg* 1979; 14:287-300.
 40. Robboy SJ, Miller T, Donahoe PK, et al. Dysgenesis of testicular and streak gonads in the syndrome of mixed gonadal dysgenesis: perspective derived from a clinicopathologic analysis of twenty-one cases. *Hum Pathol* 1982;13:700-16.
 41. Telvi L, Lebbar A, Del Pino O, Barbet JP, Chaussain JL. 45,X/46,XY mosaicism: report of 27 cases. *Pediatrics* 1999;104:304-8.
 42. Mroz K, Carrel L, Hunt PA. Germ cell development in the XXY mouse: evidence that X chromosome reactivation is independent of sexual differentiation. *Dev Biol* 1999; 207:229-38.
 43. Koopman P, Munsterberg A, Capel B, Vivian N, Lovell-Badge R. Expression of a candidate sex-determining gene during mouse testis differentiation. *Nature* 1990; 348:450-2.
 44. McElreavy K, Vilain E, Abbas N, et al. XY sex reversal associated with a deletion 5' to the SRY "HMG box" in the testis-determining region. *Proc Natl Acad Sci U S A* 1992; 89:11016-20.
 45. Wagner T, Wirth J, Meyer J, et al. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. *Cell* 1994;79: 1111-20.
 46. Ottolenghi C, Veitia R, Quintana-Murci L, et al. The region on 9p associated with 46,XY sex reversal contains several transcripts expressed in the urogenital system and a novel doublesex-related domain. *Genomics* 2000;64:170-8.
 47. De Santa Barbara P, Bonneaud N, Boizet B, et al. Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Mullerian hormone gene. *Mol Cell Biol* 1998;18:6653-65.
 48. Donahoe PK, Crawford JD, Hendren WH. True hermaphroditism: a clinical description and a proposed function for the long arm of the Y chromosome. *J Pediatr Surg* 1978;13:293-301.
 49. Nihoul-Fekete C, Lortat-Jacob S, Cachin O, Josso N. Preservation of gonadal function

- in true hermaphroditism. *J Pediatr Surg* 1984;19:50-5.
50. Berkovitz GD, Fehner PY, Marcantonio SM, et al. The role of the sex-determining region of the Y chromosome (SRY) in the etiology of 46,XX true hermaphroditism. *Hum Genet* 1992;88:411-6.
51. Torres M, Gomez-Pardo E, Dressler GR, Gruss P. Pax-2 controls multiple steps of urogenital development. *Development* 1995;121:4057-65.
52. Sanyanusin P, McNoe LA, Sullivan MJ, Weaver RG, Eccles MR. Mutation of PAX2 in two siblings with renal-coloboma syndrome. *Hum Mol Genet* 1995;4:2183-4.
53. Donahoe PK, Hendren WH. Pelvic kidney in infants and children: experience with 16 cases. *J Pediatr Surg* 1980;15:486-95.
54. Stark K, Vainio S, Vassileva G, McMahon AP. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* 1994;372:679-83.
55. Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP. Female development in mammals is regulated by Wnt-4 signalling. *Nature* 1999;397:405-9.
56. Ma L, Benson GV, Lim H, Dey SK, Maas RL. Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in mullerian duct by the synthetic estrogen diethylstilbestrol (DES). *Dev Biol* 1998;197:141-54.
57. Goodman FR, Scambler PJ. Human HOX gene mutations. *Clin Genet* 2001;59:1-11.
58. de Santa Barbara P, Roberts DJ. Tail gut endoderm and gut/genitourinary/tail development: a new tissue-specific role for Hoxa13. *Development* 2002;129:551-61.
59. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 1971;284:878-81.
60. Miller C, Degenhardt K, Sassoon DA. Fetal exposure to DES results in de-regulation of Wnt7a during uterine morphogenesis. *Nat Genet* 1998;20:228-30.
61. Belville C, Josso N, Picard JY. Persistence of Mullerian derivatives in males. *Am J Med Genet* 1999;89:218-23.
62. MacLaughlin DT, Teixeira J, Donahoe PK. Reproductive tract development — new discoveries and future directions. *Endocrinology* 2001;142:2167-72.
63. Haqq CM, Donahoe PK. Regulation of sexual dimorphism in mammals. *Physiol Rev* 1998;78:1-33.
64. Cohen-Haguenuer O, Picard JY, Mattei MG, et al. Mapping of the gene for anti-Mullerian hormone to the short arm of human chromosome 19. *Cytogenet Cell Genet* 1987;44:2-6.
65. Visser JA, McLuskey A, van Beers T, et al. Structure and chromosomal localization of the human anti-mullerian hormone type II receptor gene. *Biochem Biophys Res Commun* 1995;215:1029-36.
66. Hutson JM, Chow DW, Ng WD. Persistent Mullerian duct syndrome with transverse testicular ectopia. *Pediatr Surg Int* 1987;2:191-4.
67. Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM. Cloning of human androgen receptor complementary DNA and localization to the X chromosome. *Science* 1988;240:327-30.
68. Trapman J, Klaassen P, Kuiper GG, et al. Cloning, structure and expression of a cDNA encoding the human androgen receptor. *Biochem Biophys Res Commun* 1988;153:241-8.
69. Stocco DM. Tracking the role of a star in the sky of the new millennium. *Mol Endocrinol* 2001;15:1245-54.
70. Di Cerbo A, Biason-Laubert A, Savino M, et al. Combined 17alpha-hydroxylase/17,20-lyase deficiency caused by Phe93Cys mutation in the CYP17 gene. *J Clin Endocrinol Metab* 2002;87:898-905.
71. Morissette J, Rheume E, Leblanc JF, Luu-The V, Labrie F, Simard J. Genetic linkage mapping of HSD3B1 and HSD3B2 encoding human types I and II 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase close to D1S14 and the centromeric D1Z5 locus. *Cytogenet Cell Genet* 1995;69:59-62.
72. Peltoketo H, Luu-The V, Simard J, Adamski J. 17Beta-hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family: nomenclature and main characteristics of the 17HSD/KSR enzymes. *J Mol Endocrinol* 1999;23:1-11.
73. Andersson S, Berman DM, Jenkins EP, Russell DW. Deletion of steroid 5 alpha-reductase 2 gene in male pseudohermaphroditism. *Nature* 1991;354:159-61.
74. Jenkins EP, Hsieh CL, Milatovich A, et al. Characterization and chromosomal mapping of a human steroid 5 alpha-reductase gene and pseudogene and mapping of the mouse homologue. *Genomics* 1991;11:1102-12.
75. Donahoe PK, Crawford JD, Hendren WH. Management of neonates and children with male pseudohermaphroditism. *J Pediatr Surg* 1977;12:1045-57.
76. Lee MM, Donahoe PK, Silverman BL, et al. Measurements of serum mullerian inhibiting substance in the evaluation of children with nonpalpable gonads. *N Engl J Med* 1997;336:1480-6.
77. Lee MM, Donahoe PK, Hasegawa T, et al. Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. *J Clin Endocrinol Metab* 1996;81:571-6.
78. Rey R, Mebarki F, Forest MG, et al. Anti-Mullerian hormone in children with androgen insensitivity. *J Clin Endocrinol Metab* 1994;79:960-4.
79. Imperato-McGinley J, Zhu YS. Androgens and male physiology: the syndrome of 5alpha-reductase-2 deficiency. *Mol Cell Endocrinol* 2002;198:51-9.
80. Speiser PW. Congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Endocrinol Metab Clin North Am* 2001;30:31-59.
81. Pucholt V, Fitzsimmons JS, Gelsthorpe K, Reynolds MA, Milner RD. Location of the gene for 21-hydroxylase deficiency. *J Med Genet* 1980;17:447-52.
82. White PC, New MI, Dupont B. Molecular cloning of steroid 21-hydroxylase. *Ann N Y Acad Sci* 1985;458:277-87.
83. Merke DP, Bornstein SR, Avila NA, Chrousos GP. NIH conference: future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Ann Intern Med* 2002;136:320-34.
84. Chua SC, Szabo P, Vitek A, Grzeschik KH, John M, White PC. Cloning of cDNA encoding steroid 11 beta-hydroxylase (P450c11). *Proc Natl Acad Sci U S A* 1987;84:7193-7.
85. Mornet E, Dupont J, Vitek A, White PC. Characterization of two genes encoding human steroid 11 beta-hydroxylase (P-450(11) beta). *J Biol Chem* 1989;264:20961-7.
86. New MI. Prenatal treatment of congenital adrenal hyperplasia: author differs with technical report. *Pediatrics* 2001;107:804. [Erratum, *Pediatrics* 2001;107:1450.]
87. Schnitzer JJ, Donahoe PK. Surgical treatment of congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am* 2001;30:137-54.

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