Molecular Mechanisms of Sexual Development

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I. Introduction

Sexual determination was last discussed at Internal Medicine Grand Rounds four years ago. At that time, Jean Wilson reviewed recent data about the SRY gene, which had just been identified as the critical mediator of male sex determination. In today's Grand Rounds, I will provide an update on the role of SRY, and then will discuss several new genes that have been linked to sexual development. These studies provide a new level of understanding of the pathogenesis of human disorders of sexual determination/differentiation, and also provide a paradigm for complex developmental pathways that involve hierarchical interactions among different genes.

Sex, the biological differences between males and females, is genetically determined and results from an ordered series of developmental processes that culminate in the anatomic, reproductive, and behavioral differences that distinguish males from females. Because sexual differentiation is not essential for survival, patients with abnormalities in sexual development have provided an invaluable source of mutations that affect critical steps in these pathways. Conceptually, sexual development can be viewed as operating at two different levels. The first step, *sex determination*, directs the bipotential gonad to differentiate into a testis or an ovary; this event is determined by the presence or absence of the Y chromosome. Once this choice is made, subsequent developmental changes, termed *sexual differentiation*, lead to the sexually dimorphic characteristics of the male and female. The basic principle of sexual differentiation was defined by the classic studies of Jost and colleagues: genetic sex, determined at the time of fertilization by the presence or absence of the Y chromosome, leads to the development of testes or ovaries. Thereafter, male sexual differentiation takes place under the control of the testicular hormones testosterone and anti-Müllerian hormone, whereas female sexual differentiation takes place in their absence.

Genetic Sex → Gonadal Sex → Phenotypic Sex

Figure 1. Basic principle of mammalian sexual development

Much of our understanding of the genetics of sex determination has evolved from cytogenetic studies correlating genetic abnormalities of sexual phenotype with specific chromosomal defects. More recently, these studies have been extended by the identification of specific transcription factors that mediate key events in sex determination/differentiation.

II. Anatomy and Cell Biology of Sex Determination/Differentiation

Prior to sex determination, the gonads cannot be distinguished as testes or ovaries, and therefore are termed bipotential or indifferent. They appear as a

gonadal ridge consisting of a condensation of cells adjacent to the mesonephros. The somatic cells of these gonads originate from a region called the urogenital ridge; the primordial germ cells originate outside this region and migrate into the gonad from the stalk of the allantois. Following sexual determination, the testes and ovaries can be distinguished histologically. The testes form two distinct compartments: 1) the testicular cords, which are the precursors of the seminiferous tubules and contain the fetal Sertoli cells and the primordial germ cells; 2) the interstitial region, which surrounds the testicular cords and contains the Leydig cells and peritubular myoid cells. Analyses of chimeric mice derived from both XX and XY lineages showed that Sertoli cells are the critical site at which gonadal sex is determined (3). Following sex determination, Sertoli cells synthesize anti-Müllerian hormone (AMH, also called Müllerian-inhibiting substance), whereas Leydig cells synthesize the androgen hormone, testosterone. The presence or absence of the testes determines whether the embryo will differentiate along the male or female pathway. The ovaries, on the other hand, exhibit little evidence of structural differentiation until late in gestation, and do not actively participate in the early processes of sexual differentiation.

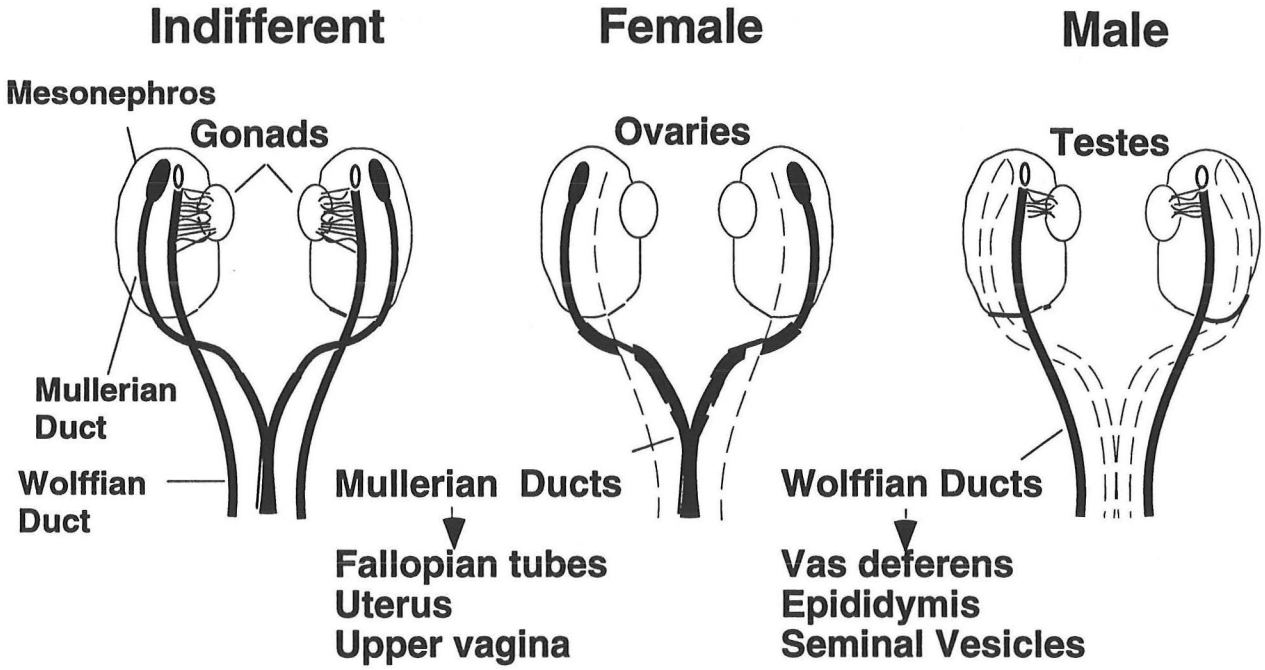


Figure 2. Origins of female and male internal genitalia from Wolffian and Müllerian ducts.

The internal genitalia derive from two sets of paired ducts--the Wolffian (mesonephric) and Müllerian ducts--which are present in the embryo at the indifferent stage. The development of the testes--and the consequent production of testosterone--causes the Wolffian ducts to differentiate into the seminal vesicles, epididymis, and vas deferens. The testes also produce AMH, which causes the Müllerian ducts to degenerate. In the absence of the testes and their products, the Wolffian ducts regress and the Müllerian ducts form the oviducts, uterus, and upper vagina in the female developmental pathway. The

external genitalia likewise derive from structures that initially are common to both sexes, including the genital tubercle, urethral folds, the urethral groove--through which the urogenital sinus empties--and the genital swellings. The 5α-dihydro derivative of testosterone promotes differentiation of the external genitalia along the male pathway, whereas no active hormonal mediators are needed for feminization of the external genitalia.

III. SRY: The Critical Determinant of Male Sex Determination

Prior to 1959, sex determination was thought to depend on the ratio of X chromosomes to autosomes. This view changed when studies of patients with abnormal numbers of sex chromosomes (XO females and XXY males) demonstrated that the Y chromosome contains a dominant developmental switch that determines sex (Ford et al. 1959; Jacobs et al. 1959; Welshons et al. 1959). These observations led to the proposal that the Y chromosome encodes a specific product, designated the testis-determining factor, that causes male sex determination. Further insights into the molecular basis for sex determination and the contribution of the Y chromosome came from studies of patients with primary sex reversal--genetic disorders in which the type of gonads present and the resulting sexual phenotype do not correlate with chromosomal sex. This phenomenon sometimes results from unequal crossing over between the X and Y chromosomes during male meiosis, as diagrammed below.

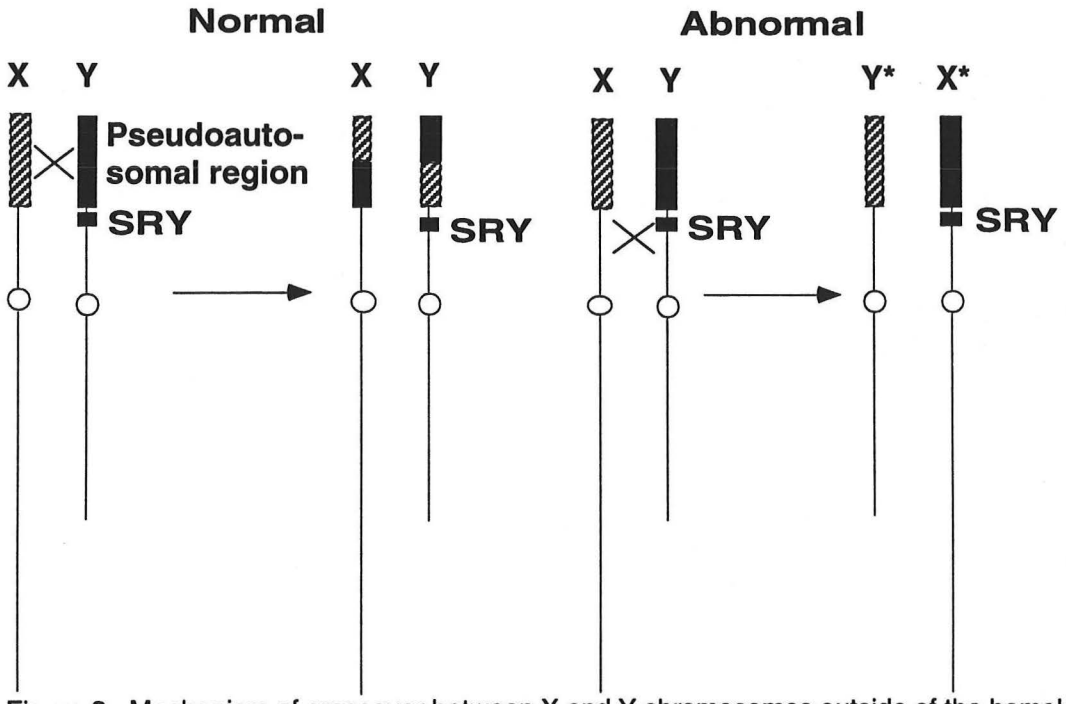


Figure 3. Mechanism of crossover between X and Y chromosomes outside of the homologous pseudoautosomal region, leading to aberrant translocation of SRY and sex-reversal. The abnormal X and Y chromosomes resulting from this translocation are designated X* and Y*.

The presence of a specific region of the Y chromosome in many XX sex-reversed males and the deletion of this region in XY sex-reversed females mapped the putative testis-determining gene to a small part of the Y chromosome proximal to the pseudoautosomal region on Yp. Subsequently, positional cloning strategies led to the isolation of a candidate gene from the Y chromosome, designated SRY (Sex-determining Region-Y chromosome; Sinclair et al. 1990), that met all criteria predicted for the testis-determining factor (reviewed by Goodfellow and Lovell-Badge 1993; Capel 1994). First, the presence or absence of this gene correlated highly with the sex-reversal phenotype, i.e., it was abnormally present in most XX patients with male phenotype and absent in a subset of XY patients with the female phenotype. More definitively, a number of sex-reversed XY patients were shown to have mutations in their *SRY* gene that precluded the expression of SRY protein (Berta et al. 1990; Jager et al. 1990). Finally, transgenic experiments in mice showed that introduction of the mouse *Sry* gene into XX mice was sufficient for testes formation and consequent male sexual differentiation (Koopman et al. 1991).

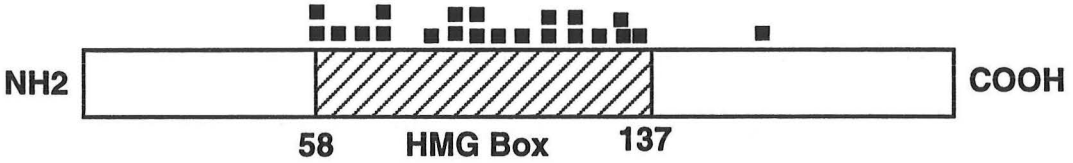


Figure 4. SRY mutations causing sex reversal. Note that almost all known mutations lie within the conserved HMG box, which mediates DNA binding.

Inspection of the SRY sequence identified one region that resembled a DNA-binding motif found in a number of other proteins called the high mobility group (HMG) box. SRY belongs to a subset of HMG box proteins that are expressed in a tissue-specific or developmentally regulated manner and that bind to specific DNA sequences. Interestingly, almost all of the known SRY mutations lie within the HMG box--the only region that is tightly conserved through evolution--supporting an essential role for DNA binding in SRY action. Based on recent multidimensional NMR spectroscopy analysis, which defined the molecular mechanism by which SRY interacts with DNA in its minor groove and induces a bend in the DNA double helix, SRY mutations causing sex reversal do not always impair DNA binding, but rather can impair DNA bending by disordering the packing of the HMG box or by impairing direct contacts with DNA (Pontiggia et al. 1994; Werner et al. 1995). Thus, SRY, like other HMG box proteins, may regulate gene expression through "architectural" effects that bend the DNA within the promoter regions, thereby facilitating the action of other transcriptional regulators.

The proven role of SRY in activating the male developmental cascade suggested that SRY activates downstream genes, which in turn mediate the conversion of the bipotential gonad into a testis; however, direct target genes of SRY have not been isolated, and its role as a regulator of gene transcription thus remains to be proven. These difficulties in demonstrating transcriptional activation by SRY, as well as analyses of phenotypically male XX patients

lacking SRY, have led to the proposal that SRY, rather than directly activating a developmental cascade, represses a negative regulator that normally inhibits the testicular pathway of development (McElreavey et al. 1993).

Based on the above discussion, it is clear that considerable gaps remain in our understanding of how SRY initiates testicular development, and hence male sexual differentiation. The possibility has been raised that the timing and levels of SRY expression are important for testes formation (Eicher and Washburn 1986). As described below, recent data with the transcription factor DAX-1 strongly support this model. Clinically, most patients with XY sex reversal do not have identifiable mutations in SRY coding sequences (Cameron and Sinclair 1997). These findings strongly suggest that other genes, in addition to SRY, are required for male sexual differentiation. As discussed below, recent studies are beginning to identify some of these genes.

IV. Other Genes Involved in Sex Determination/Differentiation

A. Steroidogenic Factor 1 (SF-1)

From the principles discussed above, it is apparent that mutations in genes that abrogate early gonadal development will cause gonadal agenesis with impaired testes formation in males and ovary formation in females, leading in either case to female sexual differentiation. One gene recently shown to play critical roles in gonadal development is that encoding SF-1 (reviewed by Parker and Schimmer 1997). SF-1, a member of the nuclear hormone receptor family of transcription factors, was first identified as an important regulator of the cytochrome P450 steroid hydroxylases. Subsequently, SF-1 was shown to regulate adrenal and gonadal expression of a number of genes required for steroidogenesis, including the cytochrome P450 steroid hydroxylases, 3ß-hydroxysteroid dehydrogenase, the ACTH receptor, and the steroidogenic acute regulatory protein. Promoter analyses demonstrated that SF-1 also activates expression of the *AMH* gene (Shen et al. 1994). Thus, SF-1 is directly involved in the production of both essential mediators of male sexual differentiation. In addition to the steroidogenic organs, SF-1 transcripts also are expressed in the ventromedial hypothalamic nucleus (VMH), a brain region implicated in reproductive behavior and appetite control, and in pituitary gonadotropes, where promoter studies again have identified SF-1 target genes that play critical roles in reproductive function (Table I).

TABLE I. Target Genes for SF-1

Gonadotropes	α-subunit of glycoprotein hormones
	Luteinizing Hormone ß
	GnRH Receptor
Adrenal cortex	Cytochrome P450 steroid hydroxylases
	3ß-hydroxysteroid dehydrogenase Type II
	Steroidogenic Acute Regulatory Protein
	ACTH receptor

	Scavenger Receptor-B1
Gonads:	
Leydig cells	Cytochrome P450 steroid hydroxylases Steroidogenic Acute Regulatory Protein
Sertoli cells	Müllerian-inhibiting Substance Aromatase
Theca and granulosa cells	Cytochrome P450 steroid hydroxylases Steroidogenic Acute Regulatory Protein Oxytocin

To define possible additional roles of SF-1 in vivo, targeted gene disruption was used to make SF-1 knockout mice. Analyses of these SF-1 knockout mice revealed essential roles of SF-1 at all three levels of the hypothalamic-pituitary-steroidogenic organ axis. Most strikingly, SF-1 knockout mice failed to develop adrenal glands and gonads, undergoing loss of the primordial organs via apoptosis at very early stages of their development (Luo et al. 1994). As a result of this, SF-1 knockout mice died shortly after birth secondary to adrenocortical insufficiency. They also exhibited male-to-female sex reversal of the internal and external genitalia, as might be expected from the degeneration of testes at developmental stages before androgens and AMH are normally produced. Finally, these SF-1-deficient mice displayed impaired function of gonadotropes (Ingraham et al. 1994; Shinoda et al. 1994), the pituitary cell type that regulates gonadal steroidogenesis, and ablation of the ventromedial hypothalamic nucleus (Ikeda et al. 1994).

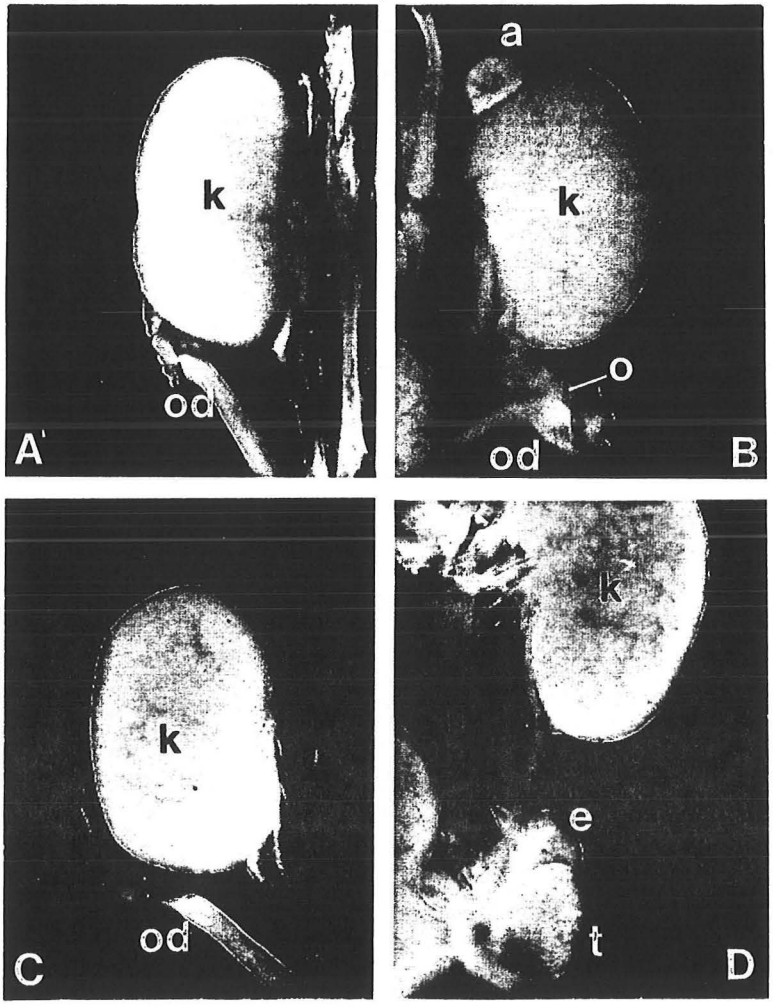


Figure 5. Newborn SF-1 knockout mice lack adrenal glands and gonads and have female internal genitalia. SF-1 knockout mice (left) and wild-type littermates (right) were sacrificed and the genitourinary tracts were dissected. A. SF-1 knockout female. B. Wild-type female. C. SF-1 knockout male. D. Wild-type male. The scale bar = 1 mm. Reprinted with permission from Luo et al. 1994. k, kidney; a, adrenal; o, ovary; t, testis; e, epididymis; od, oviduct.

These findings demonstrated unequivocally that SF-1 plays pivotal roles in the early development of the adrenal and gonadal precursors. The early embryonic expression of SF-1 and its established role as a transcription factor make it likely that SF-1 is part of the hierarchical regulatory pathway that determines the expression of downstream genes required for gonadogenesis. Although mutations in SF-1 have not yet been demonstrated in human patients, the human gene encoding SF-1 shares extensive homology with its mouse counterpart (Oba et al. 1995; Wong et al. 1995) and is expressed in many of the same sites (Ramayya et al. 1997), suggesting that SF-1 functions in humans as it does in mice. Inasmuch as the human SF-1 gene is on chromosome 9q33 (Taketo et al. 1995), it seems likely that patients will be found with abnormalities of gonadal development or sexual differentiation that map to this locus.

B. Wilms Tumor 1 (WT1)

Another gene that plays essential roles in early events in gonadogenesis is *WT1*, which was originally identified as a potential tumor suppressor gene in kindreds with familial Wilms tumors of the kidney (Call et al. 1990; Gessler et al. 1990; Haber et al. 1990). *WT1* , via alternative splicing, generates at least four different isoforms of a zinc finger, DNA binding protein that is thought to regulate gene transcription by interacting with specific DNA recognition sequences upstream of target genes. Besides differing somewhat in their preferences for DNA binding (Bickmore et al. 1992), these isoforms of WT1 also associate differentially at spliceosomes within the nucleus, suggesting that some isoforms may participate in RNA processing (Larsson et al. 1995). Analyses of sites of expression showed that WT1 is expressed in both the embryonic kidney and gonads from very early stages of development, suggesting important roles in embryogenesis.

Like SF-1, an essential role for WT1 in early gonadal development was established in studies using targeted gene disruption to make WT1 knockout mice. (Kreidberg et al. 1993). In addition to renal agenesis, these WT1 knockout mice lacked gonads, and had impaired adrenal development. As a result of their gonadal dysgenesis at early developmental stages, the internal and external genitalia developed along the female program. Although the underlying mechanisms remain to be defined, these results strongly suggest that WT1 regulates the expression of target genes in both males and females that are essential for gonadogenesis.

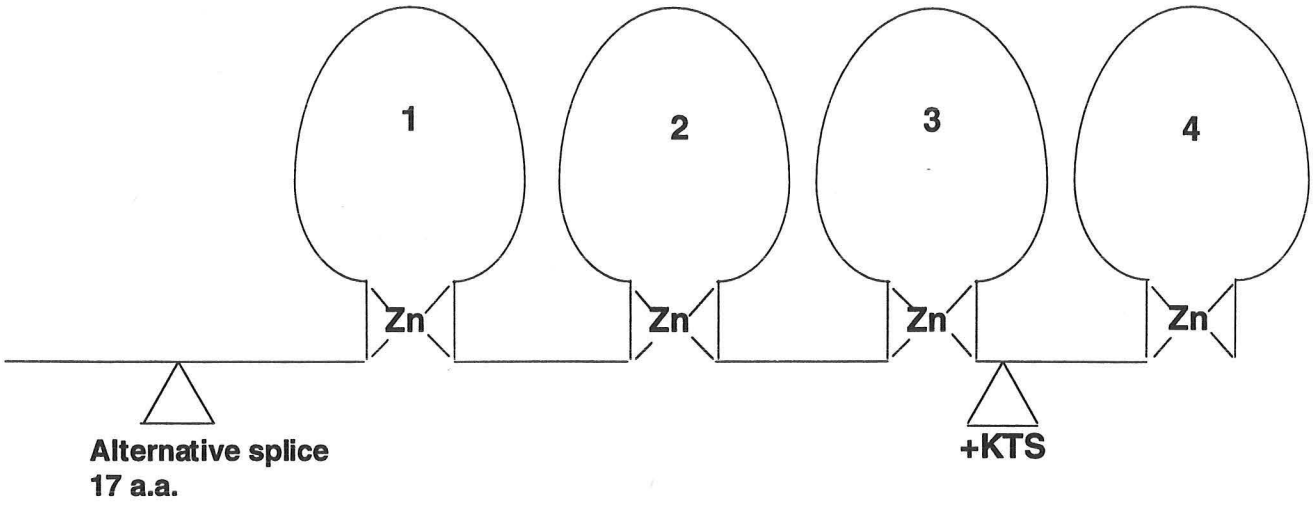


Figure 6. Functional domains of WT1. The four zinc finger DNA binding domains are indicated, as are the locations of two alternative splice sites that lead to the production of four different isoforms of WT1. Note that the +KTS splice variant disrupts the spacing between zinc fingers 3 and 4.

Mutations in WT1 have been associated with four distinct clinical syndromes.

1. Familial Wilms tumor

Although generally presenting sporadically, approximately 1% of cases of Wilms tumors occur in patients with a positive family history for this disorder. This finding led to the proposal that a tumor suppressor gene was mutated in these families in a manner akin to retinoblastoma. Efforts to map the gene(s) associated with familial Wilms tumor ultimately culminated with the isolation of the WT1 gene. These familial Wilms tumors most frequently have inherited mutations or deletions of one WT1 allele associated with somatic loss of the second allele due to gross chromosomal events. It is apparent that mutations at other chromosomal regions also are associated with familial Wilms tumor, including 11p15 (the region associated with the Beckwith-Wiedemann syndrome) and chromosome 17. These other Wilms tumor genes have not yet been identified, and remain an ongoing area of investigation.

2. WAGR Syndrome

A more encompassing phenotype associated with deletions of WT1 is the WAGR syndrome (Miller et al. 1964). This disorder, which includes Wilms tumors, Aniridia, Genitourinary abnormalities, and Mental Retardation, is associated with deletions of the region of chromosome 11 that includes the WT1 gene and other surrounding genes. The genitourinary abnormalities are relatively mild, involving cryptorchidism and hypospadias in males and horseshoe kidneys in males and females. The full phenotype reflects deletions of several genes, including WT1 and the transcription factor PAX6, mutations in which are also associated with aniridia in humans. Other genes likely lie within the chromosomal region that is deleted in WAGR, including a gene whose

expression in the embryonic brain suggests that it may contribute to the mental retardation (Schwartz et al. 1995).

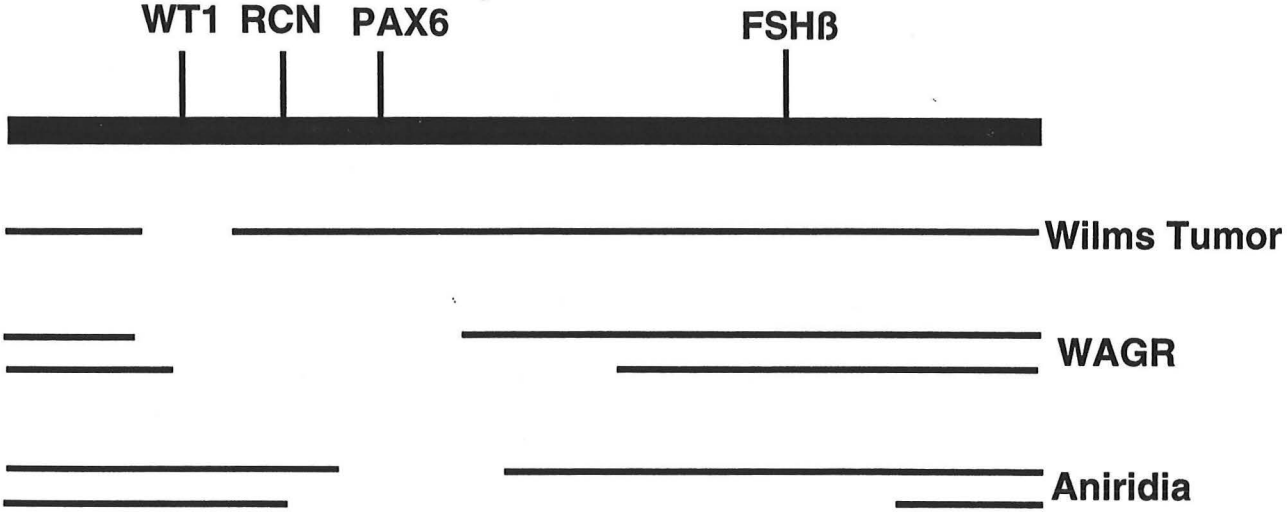


Figure 7. Contiguous genes located within the region of chromosome 11p13 that is deleted in the WAGR Syndrome. The regions that are deleted in patients have familial Wilms tumors, WAGR syndrome, and familial aniridia are indicated. FSHß, the ß-subunit of follicle-stimulating hormone; RCN, reticulocalbin.

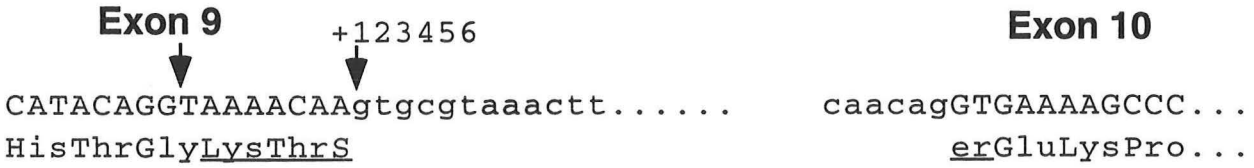
3. Denys-Drash syndrome

Denys-Drash syndrome is an autosomal dominant disorder characterized by gonadal and urogenital abnormalities in conjunction with diffuse mesangial sclerosis, with the renal disease usually presenting in the first year of life and causing end-stage renal disease by age 3. The gonadal phenotypes of these patients vary, but are generally more severe than those associated with the WAGR syndrome, with streak gonads and sex-reversal of external and internal genitalia at one extreme and varying degrees of pseudohermaphroditism in less-severely affected XY males. Wilm's tumors are often seen in families that carry Denys-Drash mutations. Almost all WT1 mutations associated with Denys-Drash syndrome are point mutations in the zinc finger DNA binding domains that abrogate DNA binding; these mutated proteins are predicted to act in a dominant negative fashion to inhibit normal function of protein encoded by the wild-type allele (Pelletier et al. 1992; Hasty et al. 1993). Based on the knockout mouse studies described above, it is very likely the inhibition of WT1 action impairs genitourinary development, with the most severe mutations leading to early gonadal dysgenesis and consequent sex reversal of external and internal genitalia.

4. Frasier syndrome

WT1 mutations also have been identified in patients with Frasier syndrome (Barbaux et al. 1997). Unlike patients with Denys-Drash syndrome, these patients do not develop Wilms tumors, but present with gonadal

dysgenesis, male pseudohermaphroditism, and focal glomerular sclerosis. Their glomerulopathy is less severe than that associated with Denys-Drash mutations, with no evidence of renal insufficiency until after age 4 and preservation of some renal function until adolescence or young adulthood. WT1 mutations that cause Frasier syndrome mutations cluster within intron 9 of the *WT1* gene, and are proposed to interfere selectively with the synthesis of the splice variant of WT1 that includes the amino acids Lysine-Threonine-Serine (+KTS) between the third and fourth zinc fingers. Although the significance of the various splice variants of WT1 is not fully understood, this finding suggests that the +KTS isoform is essential for gonadal development, but is not required to suppress the development of Wilms tumors. This finding further suggests that the same WT1 mutations that cause Frasier syndrome in boys may also cause some cases of focal glomerular sclerosis in girls, who escape diagnosis because they lack abnormalities of the external genitalia. If so, recognition of these mutations may facilitate considerably genetic counseling for family members.



Mutation	Phenotype
+2 t-c (1)	46 XY female, proteinuria (6 y), nephrotic syndrome (23 y)
+4 c-t (5)	46 XY female, proteinuria (2-5 y), nephrotic syndrome (9-18 y)
+5 g-a (4)	46 XY female, proteinuria (2-3 y), nephrotic syndrome (14 y)
+6 t-a (1)	46 XY female, nephrotic syndrome (14 y), ESRD (35 y)

Figure 8. Molecular basis for Frasier syndrome. The sequence of the *WT1* gene in the region affected by the variable +KTS splice is shown. Arrows above the sequence indicate the positions of the alternative splicing events. Intronic bases are numbered relative to the KTS exon. Specific intronic mutations associated with Frasier syndrome are shown, with the number of patients with each mutation shown in parentheses.

C. DAX-1: a potential mediator of ovarian development

1. Adrenal hypoplasia congenita

X-linked adrenal hypoplasia congenita (AHC) is a rare genetic disorder in which patients present with ACTH-insensitive adrenal insufficiency due to impaired development of the definitive zone of the adrenal cortex--the postnatal site of steroidogenesis. Histologically, these patients show varying levels of loss of normal architecture of the adrenal gland accompanied by cytomegaly and eosinophillia. If kept alive with corticosteroids, these AHC patients later frequently exhibit features of hypogonadotrophic hypogonadism, reflecting a mixed phenotype of hypothalamic and pituitary gonadotropin deficiencies that impair their ability to stimulate testicular steroidogenesis. Positional cloning efforts culminated with the isolation of a gene--designated *DAX-1* (Dosage-sensitive sex reversal, Adrenal hypoplasia congenita, X-linked). *DAX-1* encodes an atypical member of the nuclear receptor family that retains the conserved ligand binding domain but lacks the typical zinc finger DNA-binding motif (Zanaria et al. 1994), suggesting that DAX-1 is a transcription factor that regulates gene expression through protein-protein interactions. Analyses of DAX-1 in patients with AHC showed unequivocally that both impaired adrenal development and hypogonadotropic hypogonadism resulted from mutations in the same gene (Muscatelli et al. 1994); all of the mutations associated with the disorder were found in the carboxy-terminus of the protein, suggesting that residues critical for function were found in this region. Collectively, these studies strongly suggest that DAX-1, like SF-1, serves multiple roles within the hypothalamic-pituitary-steroidogenic organ axis.

2. Dosage sensitive sex-reversal

XY sex reversal is seen in some patients with a duplication of the short arm of the human X chromosome, presumably resulting from two copies of a critical gene found within a 160 kb Xp locus; this phenomenon is termed "dosage sensitive sex reversal" or DSS (Bardoni et al. 1994), and is proposed to result from a gene that interferes with the pathway of male sex determination. The identical mapping of the DAX-1 gene and the DSS region suggested that DAX-1 might antagonize the male developmental pathway in a dosage dependent manner. In support of this, recent studies have shown that overexpression of DAX-1 in transgenic mice can impair the normal male developmental pathway and cause sex reversal (Swain et al. 1998). Depending on the level of DAX-1 expression achieved, these mice exhibit gonadal changes that range from gonadal dysgenesis to production of true hermaphrodites with both testes and ovaries. It is likely, although as yet unproved, that similar imbalances between levels of expression of SRY and DAX-1 may account for some human patients with the rare intersex condition of true hermaphroditism.

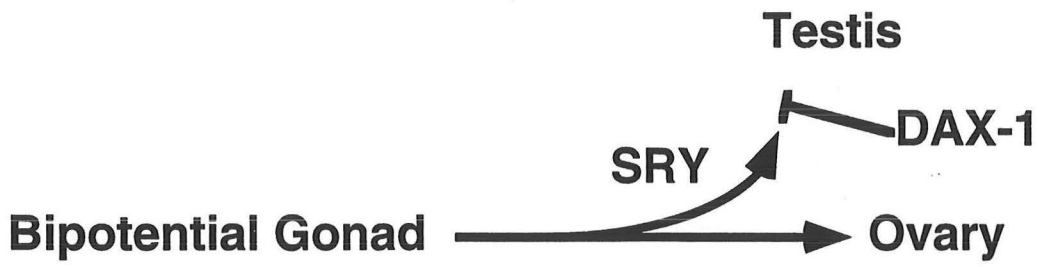


Figure 9. Model for DAX-1 antagonism of the male pathway of sex determination. Over-expression of DAX-1, as is the case in patients with duplication of the region containing the DAX-1 gene, will prevent testis formation.

The similar compound endocrine phenotypes seen in patients with DAX-1 mutations and in SF-1 knockout mice--adrenal aplasia/hypoplasia and hypogonadotrophic hypogonadism--suggest that these genes act in the same developmental pathway. Recent studies have shown that both genes are expressed in many of the same sites, including the gonads, adrenal cortex, pituitary gonadotropes, and the VMH (Swain et al. 1996; Ikeda et al. 1996). Moreover, recent studies suggest that DAX-1 can heterodimerize with SF-1, and that DAX-1 can inhibit SF-1-mediated transcriptional activation, presumably secondary to this heterodimerization (Ito et al. 1997). Alternatively, it has been proposed that DAX-1 inhibits SF-1 action by interfering with the ability of SF-1 to interact with its promoter elements upstream of SF-1 target genes. In either case, excess expression of DAX-1 is predicted to interfere with the production of AMH and androgens, thereby impeding male sexual development.

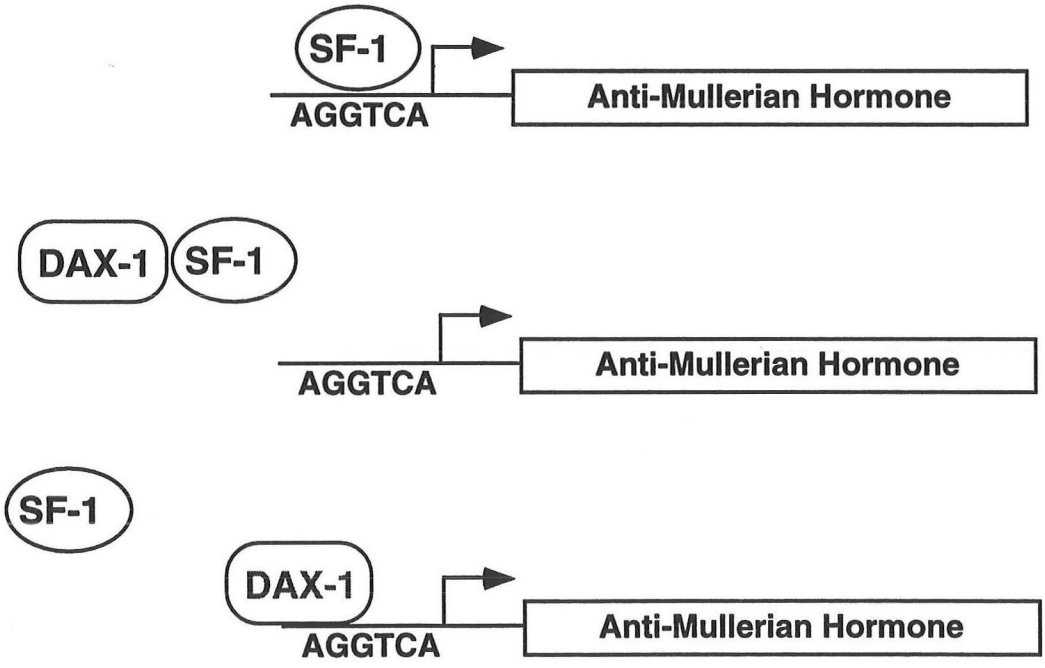


Figure 10. Models for DAX-1 antagonism of SF-1 action.

D. SOX9, a proposed mediator of testicular development

Another gene recently implicated in gonadal development is *SOX9*, an autosomal gene with homology to the DNA-binding motif of SRY. This putative gene was originally cloned because it contains an HMG box with greater than 60% homology with that of SRY (Gubbay et al. 1990). Subsequently, the *SOX9* gene, located at 17q, was shown to be mutated in patients with campomelic dysplasia (CD), a syndrome characterized by skeletal abnormalities (e.g. congenital bowing of long bones in association with brachydactyly, clinodactyly, and micrognathia) and impaired testicular development with male-to-female sex reversal (Wagner et al. 1994; Foster et al. 1994). Although originally thought to be autosomal recessive, the identification of *SOX9* as the gene responsible for CD led to the realization that most patients are heterozygotes, suggesting that haploinsufficiency leads to the clinical disorder (Cameron and Sinclair 1997). Moreover, there are differences in the degree of penetrance of the skeletal and gonadal phenotypes: whereas the vast majority of patients with CD exhibit characteristic skeletal abnormalities, only about 75% have gonadal dysgenesis and sex reversal. Sometimes, the same mutation can be associated with both CD alone and CD with sex reversal, suggesting that other genes can affect the penetrance.

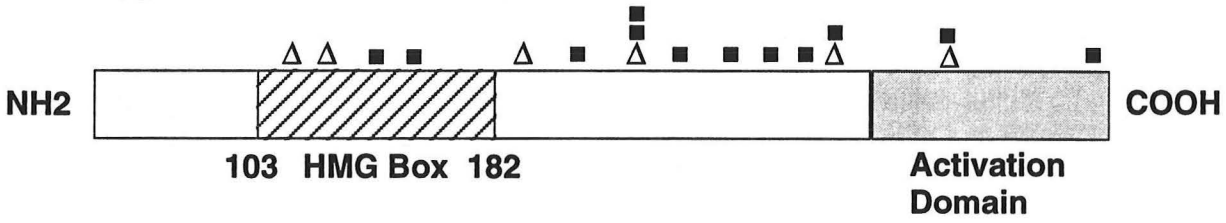


Figure 11. SOX9 mutations causing campomelic dysplasia alone (open triangles) or campomelic dysplasia with sex reversal (squares) are shown. Note that the same mutation apparently can be associated with campomelic dysplasia or campomelic dysplasia with sex reversal.

Mutations associated with CD include those that affect splicing, missense and frameshift mutations, and deletions (Cameron and Sinclair 1997). The striking preponderance of mutations in the HMG box associated with SRY is not found with SOX9, as mutations are distributed throughout the coding region. Intriguingly, in several patients with CD and sex reversal associated with balanced translocations involving the long arm of chromosome 17, the translocation breakpoint is at least 50 kilobase pairs upstream of the SOX9 gene. This finding suggests that regulatory elements considerably removed from the SOX9 promoter can affect its expression. Unlike SRY, SOX9 has both the HMG box DNA-binding domain and a transactivation domain, with some disease states resulting from deletion of a carboxy terminal transactivation domain (Südbeck et al. 1996). This finding suggests that SOX9 also participates in sex determination by activating downstream genes that are essential for testicular development. Intriguingly, SOX9 expression is much higher in fetal testes than in the ovaries, further supporting a specific role for SOX9 in testicular development and male sex determination (Kent et al. 1996; Morais de Silva et al. 1996).

V. Conclusions:

As discussed above, sexual determination and differentiation require that multiple events occur within the appropriate tissues at the appropriate times of development; defects at any of these steps can impair sexual differentiation. Although it is apparent that *SRY*, encoded by the Y chromosome, is the primary mediator of male sex determination, there remain considerable gaps in our understanding of just how SRY brings about these critical events in development. As summarized in Figure 12, it now is appreciated that both X-linked (e.g. *DAX-1*) and autosomal (e.g. *SOX9*, *SF-1*, *WT1*) genes also play critical roles in processes of sex determination and differentiation. Moreover, XY sex reversal in association with other chromosomal regions have been identified in humans (e.g. monosomy of chromosome 9q24; McDonald et al. 1997; monosomy of the long arm of chromosome 10, Wilkie et al. 1993), and in mice (Eicher et al. 1997) , implicating additional, yet-to-be-defined genes at other loci that are essential for sex determination. Critical goals of future studies are to identify the various genes that play pivotal roles in sex determination and subsequent differentiation along male or female pathways and to define the mechanisms by which these multiple genes interact. Do the genes act in hierarchical fashion, with one gene activating the expression of another? Alternatively, do certain of the genes interact directly or indirectly to induce the expression of common downstream target genes? Through a combination of additional analyses of human patients with aberrant sex development and studies in experimental model systems (e.g. knockout mice and transgenic overexpression studies), an improved understanding of these essential pathways hopefully soon will emerge.

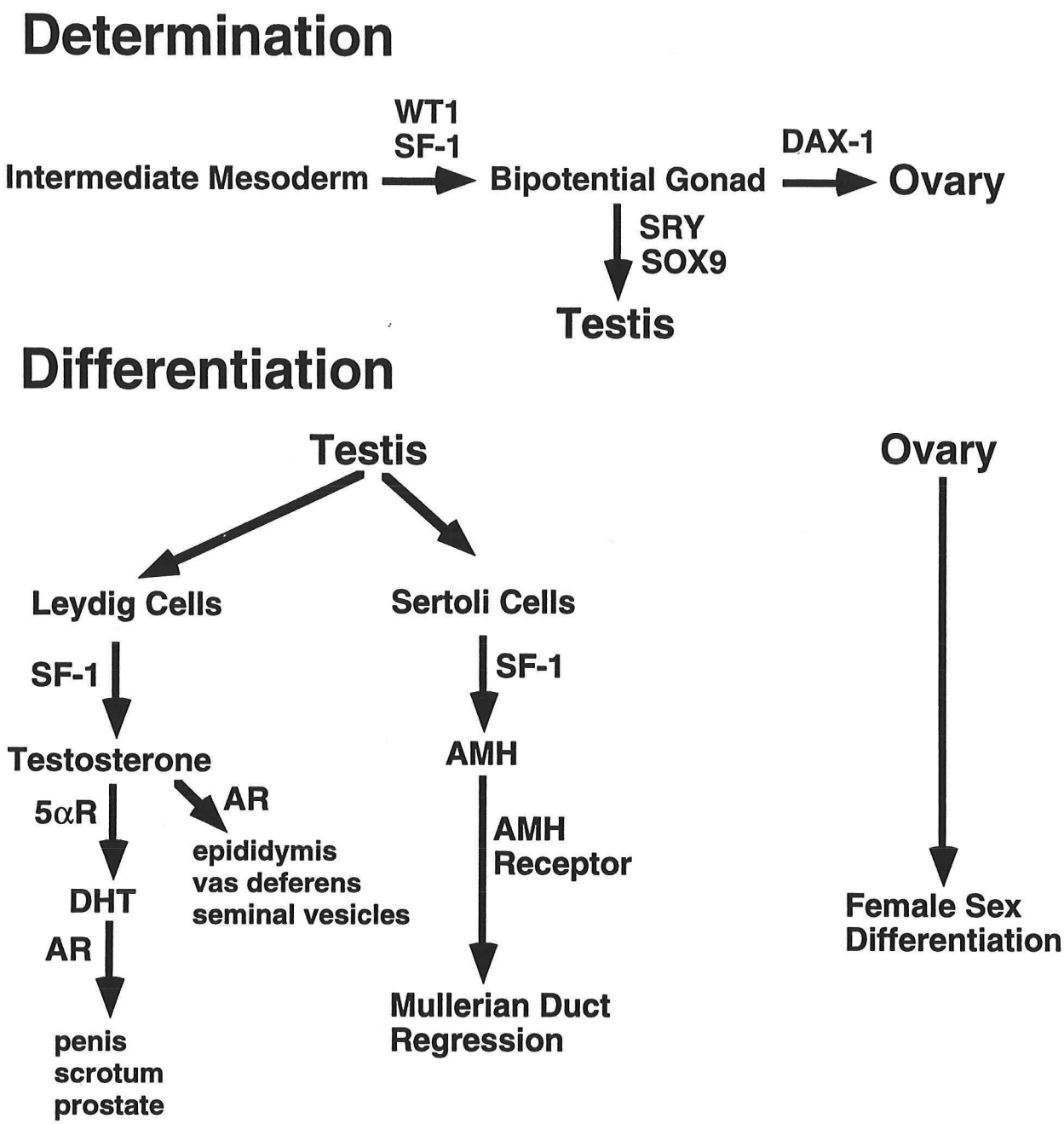


Figure 12. Overview of processes of sex determination and differentiation. The steps involved in the committment to male or female gonadal sex are illustrated, as are the steps required for sexual differentiation. DHT, dihydrotestosterone; 5αR, steroid 5α-reductase; AR, androgen receptor; AMH, Anti-Müllerian hormone.

REFERENCES

Barbaux S, Niaudet P, Gubler M-C et al. Donor splice-site mutations in WT1 are responsible for Frasier syndrome. Nature Genet 1997;17:467-470.

Bardoni B, Zanaria E, Guioli S et al. A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nature Genet 1994;7:497-501.

Berta P, Hawkins JR, Sinclair AH et al. Genetic evidence equating SRY and the testis-determining factor. Nature 1990; 348:448-450.

Bickmore WA, Oghene K, Little MH et al. Modulation of DNA binding specificity by alternative splicing of the Wilms' tumour wt1 gene transcript. Science 1992;257:235-237.

Bruening W, Bardeesy N, Silverman BL et al. Germline intronic and exonic mutations in the Wilms' tumour gene (WT1) affecting urogenital development. Nature Genet 1992;1:144-148.

Burgoyne PS, Buehr M, Koopman P, Rossant J, McLaren A. Cell-autonomous action of the testis-determining gene: Sertoli cells are exclusively XY in XX-XY chimeric mouse testes. Development 1988;102:443-450.

Call K, Glaser T, Ito C et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 1990; 60:509-520.

Cameron FJ, Sinclair AH. Mutations in SRY and SOX9: Testis-determining genes. Human Mutation 1997;9:388-395.

Capel B. The role of Sry in cellular events underlying mammalian sex determination. Curr Topics Develop Biol 1996;32:1-32.

Eicher EM, Washburn LL. Genetic control of primary sex determination in mice. Annu Rev Genet 1986;20:327-360.

Eicher EM, Washburn LL, Schork NJ et al. Sex-determining genes on mouse autosomes identified by linkage analysis of C57BL/6J-YPOS sex reversal. Nature Genet 1997;14:206-209.

Ford CE, Jones KW, Polani PE, Almeida JC, Briggs JH. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). Lancet 1959; 1:711-713.

Foster JW, Dominguez-Steglich MA, Guioli S et al. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 1994;372:525-530.

Gessler M, Poustka A, Cavenee W et al. Homozygous deletion in Wilms' tumours of a gene identified by chromosome jumping. Nature 1990;343:774-778.

Goodfellow PN, Lovell-Badge R. SRY and sex determination in mammals. Annu Rev Genet 1993;27:71-92.

Gubbay J, Collignon J, Koopman P et al. A gene mapping to the sex determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature 1990;346:245-250.

Haber DA, Buckler AJ, Glaser T et al. An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms' tumor. Cell 1990;61:1257-1269.

Hastie ND. Dominant negative mutations in the Wilms' tumour (WT1) gene cause Denys-Drash syndrome--proof that a tumour-suppressor gene plays a crucial role in normal genitourinary development. Hum Mol Genet 1993;1:293-295.

Hatano O, Takayama K, Imai T et al. Sex-dependent expression of a transcription factor, Ad4BP, regulating steroidogenic P-450 genes in the

gonads during prenatal and postnatal rat development. Development 1995;120:2787-2797.

Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL. The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. Mol Endocrinol 1995;9: 478-486.

Ikeda Y, Swain A, Weber TJ et al. Steroidogenic factor 1 and Dax-1 co-localize in multiple cell lineages: potential links in endocrine development. Mol Endocrinol 1996;10:1261-1272.

Ingraham HA, Lala DS, Ikeda Y et al. The nuclear receptor steroidogenic factor 1 acts at multiple levels of the reproductive axis. Genes Dev 1994; 8:2302-2312.

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;17:1476-1483.

Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determinating mechanism. Nature 1959;183:302-303.

Jager RJ, Anvret M, Hall K, Scherer G. A human XY female with a frame shift mutation in the candidate testis-determining gene *SRY*. Nature 1990; 348:452-454.

Jost A. Studies on sex differentiation in mammals. Recent Prog Horm Res 1953;8:379-418.

Jost A, Vigier B, Prepin J, Perchellet J. Studies on sex differentiation in mammals. Recent Prog Horm Res 1973;29:1-41.

Kent J, Wheatley SC, Andrews JE, Sinclair AH, Koopman P. A male-specific role for SOX9 in vertebrate sex determination. Development 1996; 122:2813-2822.

Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for Sry. Nature 1991; 351:117-121.

Kreidberg JA, Sariola H, Loring JM et al. WT-1 is required for early kidney development. Cell 1993;74:679-691.

Larsson SH, Charlieu J-P, Miyagawa K et al. Subnuclear localization of WT1 in splicing or transcription factor domains is regulated by alternative splicing. Cell 1995; 391-401.

Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and for male sexual differentiation. Cell 1994;77:481-490.

McDonald MT, Flejter W, Sheldon S, Putzi MJ, Gorski JL. XY sex reversal and gonadal dysgenesis due to 9p24 monosomy. Am J Med Genet 1997; 73:321-326.

McElreavey K, Vilain E, Abbas N, Herskowitz I, Fellous M. A regulatory cascade hypothesis for mammalian sex determination: SRY represses a negative regulator of male development. Proc Natl Acad Sci USA 1993; 90:3368-3372.

Miller RW, Fraumeni JF, Manning MD. Association of Wilms' tumor with aniridia, hemi-hypertrophy and other congenital anomalies. N Eng J Med 1964; 270-922-927.

Morais da Silva S, Hacker A, Harley V, Goodfellow P, Swain A, Lovell-Badge R. Sox9 expression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. Nature Genet 1996;14:62-68.

Muscatelli F, Strom TM, Walker AP et al. Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotrophic hypogonadism. Nature 1994;372:672-676.

Oba K, Yanase T, Nomura M, Morohashi K, Takayanagi R, Nawata H. Structural characterization of human Ad4BP (SF-1) gene. Biochem Biophys Res Commun 1996;226:261-267.

Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocrine Rev 1997;18:361-377.

Pelletier J, Bruening W, Kashtan CI et al. Germline mutations in the Wilms' tumor suppressor gene are associated with abnormal urogenital development in Denys-Drash syndrome. Cell 1991;67:437-447.

Pevny LH, Lovell-Badge. Sox genes find their feet. Curr Opin Genet Devel 1997;7:338-344.

Pontiggia A, Rimini R, Harley VR, Goodfellow PN, Lovell-Badge R, Bianchi ME. Sex reversing mutations affecting the architecture of SRY-DNA complexes. EMBO J 1994;13:6115-6124.

Ramayya MS, Zhou J, Kino T, Segars JH, Bondy CA, Chrousos GP. Steroidogenic factor 1 messenger ribonucleic acid expression in steroidogenic and nonsteroidogenic human tissues: Northern blot and in situ hybridization studies. J Clin Endocrinol Metab 1997;82:1799-1806.

Sadovsky Y, Crawford PA, Woodson KG et al. Mice deficient in the orphan receptor steroidogenic factor 1 lack adrenal glands and gonads but express P450 side-chain-cleavage enzyme in the placenta and have normal embryonic serum levels of corticosteroids. Proc Natl Acad Sci USA 1995;92: 10939-10943.

Schwartz F, Eisenman R, Knoll J, Gessler M, Bruns G. cDNA sequence, genomic organization, and evolutionary conservation of a novel gene from the WAGR region. Genomics 1995; 29:526-532.

Shen W-H, Moore CCD, Ikeda Y, Parker KL, Ingraham HA. Nuclear receptor steroidogenic factor 1 regulates MIS gene expression: a link to the sex determination cascade. Cell 1994;77:651-661.

Shinoda K, Lei H, Yoshii H et al. Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1-disrupted mice. Devel Dynamics 1995;204: 22-29.

Sinclair AH, Berta P, Palmer MS et al. A gene from the sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 1990;346:240-244.

Südbeck P, Schmitz MI, Baeuerle PA, Scherer G. Sex reversal by loss of the C-terminal transactivation domain of human SOX9. Nature Genet 1996; 13:230-232.

Swain A, Narvaez V, burgoyne P, Camerino G, Lovell-Badge R. Dax1 antagonizes Sry action in mammalian sex determination. Nature 1998; 391:761-767.

Swain A, Zanaria E, Hacker A, Lovell-Badge R, Camerino G. Mouse Dax-1 expression is consistent with a role in sex determination as well as in adrenal and hypothalamus function. Nature Genet 1996;12:404-409.

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: 565-567.

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;79:1111-1120.

Welshons WJ, Russell LB. The Y chromosome as the bearer of male determining factors. Proc Natl Acad Sci USA 1959;45:560-566.

Werner MH, Huth JR, Gronenborn AM, Clore GM. Molecular basis of human 46X,Y sex reversal revealed from the three-dimensional solution structure of the human SRY-DNA complex. Cell 1995;81:705-714.

Wilkie AOM, Campbell FM, Daubeney P et al. Complete and partial XY sex reversal associated with terminal deletion of 10q: report of 2 cases and literature review. Am J Med Gen 1993;46:597-600.

Wong M, Ramayya MS, Chrousos GP, Driggers PH, Parker KL. Cloning and sequence analysis of the human gene encoding steroidogenic factor 1. J Molec Endocrinol 1996;17:139-147.

Zanaria E, Muscatelli F, Bardoni B et al. An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 1994;372: 635-641.

Zazopoulos E, Lalli E, Stocco DM, Sassone-Corsi P. DNA binding and transcriptional repression by DAX-1 blocks steroidogenesis. Nature 1997; 390:311-315.