

## Editorial: Of Mice and Men: The Tale of Steroidogenic Factor-1

In John Steinbeck's famous novel, George and Lennie dream of a simple bucolic life, living on a ranch in southern California (1). However, as foreshadowed by the poem on which the title is based, their best-laid plans go awry. In medicine, we harbor similarly simplistic views of the world, if for no other reason than to bring order to complex physiological events or to provide a framework for testing ideas. More often than not, these models are incomplete, if not altogether wrong. Nonetheless, this hypothesis-driven approach is the foundation of our scientific method. In this editorial, I will highlight the interplay between genetic models in mice and our understanding of inherited reproductive disorders in man, focusing on recent findings involving steroidogenic factor-1 (SF-1).

Increasingly, mouse models help to unravel complex physiological pathways, including the regulation of reproduction (2). Genetic studies in humans historically have been based on testing candidate genes or performing linkage studies, which are rarely applicable when reproduction is compromised. Mice provide an opportunity for performing targeted mutagenesis, including knockout and knock-in approaches, as well as transgenic expression of genes (3). More recently, ethylnitrosourea and transgenic insertional mutagenesis enable "forward genetic" approaches that use phenotypic screens to identify novel genes (4). Although we often focus on the differences in mouse and human phenotypes caused by mutations in homologous genes, it is notable that different inbred strains of mice also differ considerably in the phenotypic expression of traits; this feature potentially allows the identification of modifier genes in mice of different genetic backgrounds (5).

A few examples in the field of reproduction illustrate the conceptual advantages conferred by parallel studies in humans and in mice. This year, a report described hypogonadotropic hypogonadism caused by mutations in GPR54, a G protein-coupled seven-transmembrane receptor proposed to regulate puberty and gonadotropin production (6). Linkage studies and mutational analyses in humans were accompanied by characterization of a GPR54 knockout mouse, providing independent lines of evidence that this receptor plays a key role in reactivation of the GnRH pulse generator (6, 7). The *DAX1* (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1) gene was identified in humans with X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. A *Dax1*

knockout model in mice revealed a primary testicular defect that had been unrecognized in humans because of concomitant gonadotropin deficiency (8, 9).

A series of recent reports on SF-1 mutations in humans reveal a surprisingly broad spectrum of phenotypic features (10–15) and underscore the dose-dependent action of SF-1. What were the critical steps that brought us to this level of understanding?

SF-1 (also known as adrenal 4-binding protein, Ad4BP) was originally identified as a transcription factor that binds to DNA regulatory elements in the proximal promoter regions of steroidogenic enzyme genes (16). In fact, SF-1 is considered a master regulator of reproduction, because its targets include genes at every level of the hypothalamic-pituitary-gonadal axis, as well as most genes involved in gonadal and adrenal steroidogenesis. The cloning of bovine and mouse genes encoding Sf-1 revealed that it resembled the *Drosophila* orphan nuclear receptor, *fushi tarazu* factor-1 (Ftz), a gene involved in segmentation. The human SF-1 gene (officially designated NR5A1) encodes a 461-amino-acid protein that shares structural similarities with members of the nuclear receptor superfamily. Like other nuclear receptors, SF-1 binds to specific DNA sequences in target genes and regulates transcription. Specifically, it binds to DNA as a monomer and recognizes variations of the DNA sequence motif, T/CCA AGGTCA (17). In most cases, SF-1 functions cooperatively with other transcription factors to modulate the timing and level of gene expression. It is this cooperative interaction with other factors that allows it to regulate so many target genes, which differ widely in their expression patterns and regulation. Critical structural regions in SF-1 include a zinc finger DNA binding domain, an "A" box (or FTZF1 box) accessory DNA-binding domain, a hinge region, and an activation function-2 domain involved in transcriptional control. Unlike many classical nuclear receptors, such as the estrogen, glucocorticoid, or thyroid hormone receptors, there is no known ligand that regulates SF-1-mediated transcription. Consequently, it is classified as an orphan nuclear receptor to recognize the possibility that a ligand may yet be discovered. It is possible, however, that SF-1 is regulated independently of a specific ligand; its pattern and level of expression, interaction with other transcription factors, or posttranslational modifications represent plausible means to control its action (18). In the absence of a known ligand, how does one determine its physiological role? A first step is to characterize when and where SF-1 is expressed, potentially predicting possible functions.

In the mouse, Sf-1 is first expressed in the urogenital ridge at embryonic d 9 (e9) (19). After gonadal determination (e13), Sf-1 is expressed in a population of rapidly proliferating cells in the developing testis (20). In Sertoli cells, Sf-1 regulates Müllerian inhibiting substance expression, leading to regres-

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Abbreviations: *DAX1*, Dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1; SF-1, steroidogenic factor-1; STAR, steroidogenic acute regulatory protein; VMH, ventral medial hypothalamus.

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sion of Müllerian structures in males. In Leydig cells, Sf-1 regulates the steroidogenic enzyme genes that control testosterone biosynthesis. Sf-1 also plays an important role in the normal development and function of the hypothalamic-pituitary-gonadal axis. It is expressed in the ventral medial hypothalamus (VMH) and in pituitary gonadotropes. Thus, Sf-1 regulates an enormous number of genes involved in sex determination and differentiation, reproduction, and steroidogenesis (16).

The physiological role of Sf-1 was clearly established by generating knockout mice (21), which were found to have adrenal and gonadal agenesis. The XY mice exhibit male-to-female sex reversal, including persistent Müllerian structures, reflecting the absence of fetal androgens and Müllerian inhibiting substance. The knockout mice also have decreased levels of gonadotropins and a near absence of the VMH. Gonadotrope function could be restored by GnRH treatment, suggesting that Sf-1 deficiency does not result in a complete loss of gonadotrope cell function. These striking phenotypic features in Sf-1-deficient mice generated considerable interest because they demonstrated that orphan nuclear receptors might play important developmental roles. Targeted mutagenesis of *Sf1* has now been performed in the VMH, gonadotropes, and Leydig cells, confirming its intrinsic function in these tissues (22).

The mouse Sf-1 knockout phenotype prompted studies of humans with combined adrenal and gonadal defects for possible mutations in SF-1 (10). There are now five reported human SF-1 mutations, including two reports in recent issues of *The Journal of Clinical Endocrinology & Metabolism* (14, 15). Each new case provides important insight into the physiological role of SF-1 and clarifies the phenotypic spectrum associated with SF-1 mutations (see Table 2 in Ref. 14). The first human SF-1 mutation (G35E) was described in a patient with primary adrenal failure, XY sex reversal and persistent Müllerian structures (10), thereby exhibiting features very similar to the knockout mouse. Unexpectedly, however, DNA sequencing revealed a heterozygous mutation in the DNA binding domain. Thus, in humans, a heterozygous mutation in SF-1 appeared to cause the same phenotypic consequences as complete deficiency of Sf-1 in the mouse. These clinical effects were thought to primarily result from a loss-of-function effect, although it is difficult to exclude the possibility that the mutant protein exerts some degree of dominant negative activity, inhibiting the function of SF-1 expressed from the normal allele (23). The concept of dose-dependent action of SF-1 was reinforced by the second reported case, also an XY female with adrenal insufficiency. This patient was homozygous for an SF-1 mutation (R92Q) that only partially impaired DNA binding (11). Of note, three heterozygous relatives (both parents and a sibling) were clinically normal, with no evidence of adrenal insufficiency or reproductive dysfunction. Thus, this milder SF-1 mutation did not cause phenotypic effects unless transmitted on both alleles. Two recently reported cases describe patients with heterozygous SF-1 mutations that generate null alleles since almost all of the protein is missing (14, 15). Both of these XY individuals have testicular dysgenesis but normal adrenal function. Similarly, an XY patient with a heterozygous mutation reported to exert dominant negative activity, had tes-

ticular regression but normal adrenal function (13). These patients differ in the extent of virilization and regression of Müllerian structures, suggesting variability in Leydig and Sertoli cell function. Thus, in humans, the testis may be more sensitive to loss of SF-1 function than the adrenal gland, or the adrenal gland may have a greater capacity to undergo compensatory growth and function. Of note, these gland-specific sensitivities are different in heterozygous Sf-1 knockout mice; testis development is relatively normal but the mice have adrenal hypoplasia and blunted stress-induced cortisol responses (24, 25). Only one SF-1 mutation has been reported in a prepubertal XX individual who presented with adrenal insufficiency (12). Although there was evidence that ovarian tissue was present, the consequences of the SF-1 mutation on adult ovarian function are currently unknown.

When confronted with patients who have adrenal insufficiency and gonadal dysfunction, the differential diagnosis has become surprising broad. Hypopituitarism can usually be excluded based on routine hormone testing. Mutations in STAR (steroidogenic acute regulatory protein) are recessive and cause lipoid adrenal hyperplasia (26). XY subjects have adrenal insufficiency and Leydig cell failure because of defective cholesterol transport and toxic accumulation of lipid precursors, resulting in undervirilized or female genitalia. XX individuals have adrenal dysfunction and impaired ovarian function but relatively normal feminization because ovarian steroid synthesis is preserved initially and the accumulation of steroid precursors is reduced because follicle development is critical (26). The phenotype of patients with STAR mutations is similar to those with mutations in CYP11A1 (side-chain cleavage enzyme), which cause a block in the synthesis of all downstream steroids (27). Mutations in DAX-1 cause X-linked adrenal hypoplasia congenita, which is characterized by adrenal insufficiency, hypogonadotropic hypogonadism, and testicular dysgenesis (9). As noted above, mutations in SF-1 cause a phenotypic spectrum that can include variable degrees of adrenal insufficiency and testicular dysgenesis.

The recent observation that some patients with SF-1 mutations have testicular dysgenesis without adrenal insufficiency raises the issue of which genetic abnormalities should be considered in XY patients with gonadal dysgenesis. Some of this population will have Klinefelter's syndrome (XXY and variants); others may have XO/XY mosaicism. In principle, many genes involved in testis determination and differentiation are candidates for causing XY testicular dysgenesis (28). Mutations in sex-determining region on the Y chromosome preempt testis development but only account for about 10–15% of cases of XY sex reversal (29). Mutations in Wilm's tumor-1 or Sry-related high mobility group box cause gonadal dysgenesis but are also associated with renal or skeletal abnormalities, respectively. Duplications of *WNT4* (wingless-type mouse mammary tumor virus integration site family, member 4), or *DAX1* can cause testis dysgenesis. Genes involved in Leydig cell development [*DHH* (Desert Hedgehog), *ARX* (Aristaless-related homeobox, X-linked), *LHCGR* (LH receptor)], or androgen synthesis (*STAR*, *CYP11A1*, *CYP17*, *HSD3B2*, *SRD5A2*) prevent normal virilization and can be associated with or mimic testicular dysgenesis. Likewise, defects in androgen action, via the andro-

gen receptor, cause a wide range of undervirilization because of androgen insensitivity. It is useful to distinguish the clinical features of Sertoli cell and Leydig cell dysfunction. If Müllerian regression has occurred, Sertoli cell differentiation and function must have been relatively normal, at least during fetal development. On the other hand, the extent of virilization reflects Leydig cell differentiation, androgen synthesis, and action.

In summary, there has been considerable progress in the identification of genetic mutations associated with XY gonadal dysgenesis. Increasingly, these candidates are identified based on phenotypes observed in knockout mice. The important role of gene dosage is a recurring observation for pathways that regulate gonadal development. This feature is dramatically illustrated by mutations in SF-1, which cause a spectrum of phenotypes associated with graded loss of SF-1 function. It is likely that these phenotypes are also influenced by genetic background or modifier genes. This concept can be explored further by identifying additional patients with SF-1 mutations and by examining the effects of Sf-1 mutations in mice of different genetic backgrounds. In practical terms, these recent reports indicate that mutations in SF-1 should be considered in patients with XY gonadal dysgenesis, whether or not there is evidence of adrenal insufficiency (14, 15).

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### Acknowledgments

Received October 15, 2004. Accepted October 17, 2004.

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This work was supported by National Institutes of Health Grants HD044801 and HD043425.

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