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SF1 MUTATION IN HUMANS

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INTRODUCTION

The purpose of this lead article is to bring readers up to date on the phenotypes, genotypes, and pathogenesis of the steroidogenic factor (SF)1 mutation that pediatric endocrinologists encounter in their practices and to provide new insights into SF1 function in humans. Steroidogenic factor 1 (Sf1 in mice or SF1 in humans), also called Ad4BP or NR5A1, is a nuclear transcriptional factor that binds to target gene promoters as a monomer and recognizes a canonical half-site motif. Structurally, both Sf1 and SF1 have characteristic domains

of nuclear transcriptional factors. These consist of a zinc finger DNA-binding domain, a ligand-binding domain, and an activation function-2 domain. There is also an accessory DNA-binding domain that confers binding site stability and specificity.

Originally, SF1 was isolated as a global regulator for P450 steroid hydroxylases.^{1,2} SF1 was thought to be responsible for tissue-specific expression of these enzymes in the adrenals and gonads. Subsequent studies in vitro have shown that Sf1 and SF1 regulate a lot of genes involved in adrenal and gonadal development, sex differentiation, steroidogenesis, reproduction, and many other metabolic functions.^{2,3} Thus, Sf1 and SF1 play pivotal roles in the development and function of multiple endocrine organs.

From The Editor's Desk

This issue of *GGH* Volume 24, Number 1 is only available on-line and will be not be printed and mailed due to budgetary constraints. However this issue is available either as a PDF file or a web page so you can file it and/or print it and keep it for your enjoyment and as a reference resource.

The current issue includes an excellent and timely review of the "SF1 Mutations in Humans" by Dr. Tomonobu Hasegawa, plus 19 reviews of current papers in the literature with comments by the editorial board. There are four reviews pertaining to growth hormone treatment including the consensus guidelines of adult growth hormone deficiency, two addressing growth of celiac patients, three pertaining to height related issues on quality of life, the in vitro fertilization children or the genetics of stature. There are also two reviews regarding the aortic dilatation and the uterine development of Turner patients. In addition the late effects on cancer survivors, hypopituitarism following traumatic brain injury, and diabetes and stroke in hypopituitarism are also reviewed. I also want to bring to your attention the reviews on the FTO gene in obesity and the monoallelic expression of autosomal genes. Finally there are two reviews of papers dealing with two frequent alterations in pediatric endocrine practices, namely metabolic syndrome in brothers of PCOS women and the ventricular function of congenital hypothyroidism in neonates.

The economic situation in the country is being reflected in our journal. The reduced funding for continuous medical education will only allow us to publish two electronic issues in 2008, unless there is a renewed commitment for sponsorships that will allow us to provide our readers with a high quality journal more frequently. We will continue to search for means and will appreciate your tax deductible contributions. You may do so on line (www.GGHjournal.com or PedsAcademics.org) and click *make a donation*, or you may send a check to Pediatric Sunshine Academics, 1040 Alston Rd., Santa Barbara, CA 93108.

Thank you for your support,
Fima Lifshitz, MD
Editor-in-Chief

The murine Sf1 also orchestrates the development and function of multiple endocrine organs *in vivo*, judging from the striking, but complex phenotypes of its knockout mice. The Sf1 knockout mice showed adrenal and gonadal agenesis, impaired function of pituitary gonadotropes, and structural abnormalities of ventromedial hypothalamic nucleus (VMH).^{2,4} All knockout mice died within 2 weeks due to adrenal insufficiency. Moreover, recently established tissue- or cell-specific Sf1 knockout mice clearly demonstrated *in vivo* the direct and pivotal function of Sf1 in Leydig cells, granulosa cells, pituitary gonadotropes, and VMH.⁵⁻⁷

PHENOTYPES, GENOTYPES, AND PATHOGENESIS OF SF1 MUTATION IN HUMANS

The critical role of murine Sf1 *in vivo* strongly suggests the importance of SF1 in humans, prompting endocrinologists to identify patients with SF1 mutations. Initially, the rare 46,XY patients that showed severe gonadal dysgenesis together with primary adrenal failure were the main focus to identify SF1 mutations. These alterations were analogous to the phenotypes of the knockout mice. Indeed, the first described human patient with SF1 mutation (a heterozygous G35E) was a 46,XY female who presented with primary adrenal failure in the first 2 weeks of life; she had a vascular collapse at 17 days of age. The phenotype of this patient was similar to those seen in Sf1 knockout mice, albeit less severe. This patient's serum cortisol was 1.2 mcg/dL and aldosterone was 5.0 ng/dL, both of which were quite low considering the clinical condition, together with a high plasma ACTH (1,165 pg/mL). She had been treated with glucocorticoids

and mineralocorticoids. Before the induction of puberty as female, her pituitary gonadotropins responded to GnRH stimulation test: LH (1.2 → 8.6 mIU/mL) and FSH (17.8 → 38.0 mIU/mL). No response of testosterone was observed by hCG stimulation test. At laparotomy, normal Mullerian structures and streak-like gonads were found. Histological examination of the gonads showed poorly differentiated tubules and connective tissue. Mutation analysis of SF1 revealed a heterozygous mutation in the proximal box (P-box) of the first zinc finger of SF1. The P-box is important for the recognition of DNA binding and confers specificity to nuclear receptors in the regulation of target genes. The mutant SF1 protein did not bind to a canonical SF1 binding site, did not transactivate the SF1 responsible gene, and did not exhibit dominant-negative effects.⁸

The next reported 46,XY patient with SF1 mutation (homozygous R92Q) was a normal female baby who presented one day after birth with a hypoglycemic convulsion due to primary adrenal failure.⁹ Thereafter, the phenotypic spectrum of the SF1 mutation in humans has been strikingly expanded.¹⁰⁻¹⁸ The phenotypes, genotypes, and pathogenesis of SF1 mutation in humans reported to date are summarized in Tables 1-3. A number of "milder form" 46,XY patients have also been reported. These patients had 46,XY disorders of sex development (DSD), namely, testicular dysgenesis (or impaired androgen production) with normal adrenal function. Six 46,XX subjects have been reported, all of whom had seemingly normal ovarian development and function; one out of the 6 had primary adrenal failure.

Table 1. Reported Cases of SF1 Mutation in Humans

Case	1	2	3	4	5	6
Age (years)	20	newborn	31	6	27	newborn
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY
Legal sex	Female	Female	Female	Female	Female	Female
Mutation	G35E/wild	R92/R92	1058-1065del/wild	C16x/wild	18delC/wild	V15M/wild
Clinical Features						
External genitalia	Normal female	Normal female	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Clitoromegaly	Normal female
Gonadal histology	Testicular dysgenesis	Not described	Testicular regression	Testicular dysgenesis	Testicular dysgenesis	Testis
Adrenal failure	Yes	Yes	No	No	No	No
Obesity	Yes	NA	Yes	Yes	Yes	NA
Sf-1 function of mutant allele (%)	0	0-50	0	0	0	0
Dominant negative effect of mutant allele	No	Not described	Yes	No	No	No
Total SF-1 function <i>in vivo</i> (%)	50	<50	0-50	50	50	50
Reference	8	9	10	11	12	13

These phenotypes in 46,XX subjects suggested sexual dimorphism in SF1 function in gonads.

THE IMPORTANT ROLE OF SF1 GENE DOSAGE

Heterozygous mutation of SF1 causing human disease has established the concept of a dose-dependent action of SF1 in vivo. In contrast, heterozygous Sf1 knockout mice show no variations in phenotype, although latent adrenal insufficiency has been unmasked under stressful conditions.¹⁹ Nineteen identified patients are listed in Tables 1-3. All reported patients except case 2 had a heterozygous mutation of the SF1 gene. In cases 1 and also in cases 4 to 11, the mutant SF1 had null function without dominant negative effects. Thus, all of these 9 patients had 50% of total SF1 function in vivo. In cases 12 to 15, the mutant SF1 had null to 20% function. These 4 patients had 50% to 60% of total SF1 function. In case 18, the mutant SF1 only had a 55% function without dominant negative effects, suggesting that this patient had 77.5% of total SF1 function. It was of note that case 2 had a homozygous mutation of SF1. This patient had less than 50% of total SF-1 function in vivo, judging from the mutant SF-1 with 0% to 50% of total SF1 function. On the other hand, her parents and a brother had heterozygous mutations, thus these 3 members of the patient's family were phenotypically normal and had more than 50% of total SF1 function. Case 3 had heterozygous mutation. This mutation had null function together with dominant-negative effect. Therefore, case 3 had less than 50% of total SF1 function in vivo. Taken together, these

reported patients have established the importance of dosage-dependent action of SF1 in humans.

"MILDER FORM" OF 46,XY PATIENTS

Sixteen out of 18 of the 46,XY patients reported were reared as female from birth (case 18 was suspected to have 46,XY although the karyotype was not described). Androgen production in fetal testis must therefore be insufficient. Four patients showed testicular dysgenesis or regression on macroscopic or microscopic examination. Conversely, only 2 patients (cases 1 and 2) showed adrenal failure. This suggests that in humans the testis might be more sensitive to a partial loss of SF1 function than the adrenal gland.

We described a 27-year-old Japanese woman with testicular dysgenesis without adrenal failure.¹² This woman never had an adrenal crisis, even at the time of infection. She had clitoromegaly, advanced virilization during pubertal age (such as voice breakage and hirsutism), and primary amenorrhea. Her karyotype was 46,XY. Small masses were palpable bilaterally in the inguinal regions. Skin pigmentation was not observed and her plasma ACTH (21 pg/mL) and serum cortisol (13.4 mcg/dL) were normal. An ACTH stimulation test showed a normal response of cortisol (25.3 mcg/dL). Urine steroid profile by a gas liquid chromatograph/mass spectrometry indicated normal steroidogenic enzyme activities. Bilateral gonadectomy was performed, and histological examination of the gonads showed

Table 2. Reported Cases of SF1 Mutation in Humans

Case	7	8	9	10	11	12
Age (years)	newborn	newborn	newborn	2	2	22
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY
Legal sex	Female	Female	Male	Female	Female	Female
Mutation	M78I/wild	G91S/wild	L437Q/wild	C55/wild	Delta395E/wild	R84C/wild
Clinical Features						
External genitalia	Normal female	Clitoromegaly urogenital sinus	Small phallus hypospadias chordee	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Slight clitoromegaly posterior labial fusion
Gonadal histology	Testis	Testis	Testis	Testis	Testis	Testis
Adrenal failure	No	No	No	No	No	No
Obesity	NA	NA	NA	NA	NA	NA
Sf-1 function of mutant allele (%)	0	0	0	0	0	10
Dominant negative effect of mutant allele	No	No	No	No	No	NA
Total SF-1 function in vivo (%)	50	50	50	50	50	55
Reference	13	13	13	14	14	15

Remarks

Case 7 Mother has M78I/wild
Case 8 Mother has G91S/wild

dysgenetic testes, severely hyalinized seminiferous tubules containing a few Sertoli cells, and loose interstitium containing a few Leydig cells. Molecular analysis of SF1 revealed a heterozygous single base pair deletion (18delC), theoretically leading to frameshift and early termination. Indeed, mutant SF1 failed to activate the target gene in transactivation analysis and did not have a dominant-negative effect.

The presence of “milder form” of 46,XY patients were again in contrast with XY heterozygous mice, the Sf1 knockout allele showed normal external genitalia, normal fertility, but latent adrenal insufficiency under stressful conditions.¹⁹ Thus, species differences between mice and humans exist in terms of phenotypes due to loss of function of Sf1 or SF1.

SEEMINGLY NORMAL OVARIAN DEVELOPMENT AND FUNCTION IN 46,XX PATIENTS

In humans, there have been 6 cases of 46,XX reported with SF1 mutation. All 6 had seemingly normal ovarian development and function. Only one had primary adrenal failure. Case 20 was the first reported 46,XX patient with SF1 mutation.¹⁸ This phenotypically normal 14-month-old girl developed adrenal insufficiency and seizures after otitis and tonsillitis. At that time, hyponatremia (serum Na 104 mmol/L), hyperkalemia (serum K 8.0 mmol/L), elevated plasma ACTH (2,200 pg/mL), and inappropriately

low cortisol (165 nmol/L) indicated primary adrenal insufficiency. Serum LH and FSH were 0.5 mIU/mL and 2.8 mIU/mL at the age of 14 and 27 months, respectively. Imaging studies using pelvic ultrasonography and MRI confirmed the presence of bilateral ovaries of normal size. Thus, no evidence of abnormality of ovarian development and function were found.

Recently, the mothers of cases 7, 8, 12, 16, and 18 were reported to have the same mutation that was detected in the patients, indicating the ovarian development and function of these mothers were completely normal. Moreover, none of the mothers showed adrenal insufficiency. These 5 families suggested a sex-limited autosomal dominant inheritance of the SF1 mutation.

OBESITY IN ADULT PATIENTS

Four out of 5 of the 46,XY adult patients with an SF1 mutation had obesity. Thus, obesity might be part of the phenotype of SF1 mutation in humans. A partial loss of SF1 function in the VMH in humans may lead to obesity. The presence of obesity was consistent with mice studies. Majdic et al²⁰ rescued Sf1 knockout mice with corticosteroid injections, followed by adrenal gland transplantation. These transplanted mice had indistinguishable ACTH and corticosterone levels to wild-type mice, indicating restoration of hypothalamic-pituitary-adrenal axis. With gonadectomy, at earlier ages

Table 3. Reported Cases of SF1 Mutation in Humans

Case	13	14	15	16	17	18	19
Age (years)	4	14	10	8	22	NA	1
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	NA	46,XX
Legal sex	Female	Female	Female	Female	Female	Male	Female
Mutation	C33S/wild	R84H/wild	Y138X/wild	c1277dupT/wild	C424_427dupCCCA/wild	V333M/wild	R255L/wild
Clinical Features							
External genitalia	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Clitoromegaly urogenital sinus	Normal female	Micro-penis anorchia	Normal female
Gonadal histology	Testis	Testis	Testis	Testis	Streak	Fibrous tissue	Ovary
Adrenal failure	No	No	No	No	No	No	Yes
Obesity	NA	NA	NA	NA	NA	NA	NA
Sf-1 function of mutant allele (%)	0-20	0-20	0-20	NA	NA	55	0
Dominant negative effect of mutant allele	No	No	No	NA	NA	No	No
Total SF-1 function in vivo (%)	50-60	50-60	50-60	NA	NA	77.5	50
Reference	16	16	16	16	16	17	18

Remarks

Case 16 Mother has c1277dupT/wild

Case 18 Mother has V335M/wild Phenotypically normal dizygotic twin brother has V355M/wild

the weights of transplanted mice did not differ significantly from the wild-type mice. Later in life, adrenal-transplanted Sf1 knockout mice developed obesity due to decreased spontaneous locomotor activity, rather than increased appetite. It was of note that obesity was considerably more severe in females, although the reason for this sexual difference was unknown. Sf1 and the VMH nucleus in the hypothalamus were thought to play important roles in metabolism rather than in appetite regulation.

Increased weight also occurred in CNS-specific Sf1 knockout mice fed a high-fat diet.⁷ Considering the Sf1 expression in CNS, the responsible region of obesity must be VMH. CNS-specific Sf1 knockout mice showed decreased wheel running capacity before becoming obese, indicating that obesity was due to decreased spontaneous locomotor activity.

Brain-derived neurotrophic factor (Bdnf), which stimulates growth of neurons via TrkB receptor, is expressed in the hypothalamus including the VMH. CNS-specific Bdnf knockout mice also became obese. This raised the question of whether Bdnf was a direct target gene of Sf1 in VMH. However, CNS-specific Sf1 knockout mice developed obesity only when ingesting a high-fat diet, while adrenal-transplanted Sf1 knockout mice showed obesity when fed a regular diet. Some plausible explanations are possible for these differences. Subtle abnormalities in function of adrenal transplants in original Sf1 knockout mice may result in glucocorticoid excess. The presence of gonads in CNS-specific Sf1 knockout mice may ameliorate the effects of sex steroid deficiency. It should also be kept in mind that Cre-mediated disruption of Sf1 in CNS-specific Sf1 knockout mice at ~E14 may permit certain developmental events to occur before inactivation, in contrast to original Sf1 knockout mice. Most patients with SF1 mutation were children at the time of the study, thus long-term follow-up is necessary to ascertain if they develop obesity as adults.

UNSOLVED ISSUES OF SF1 MUTATION IN HUMANS

Some important issues regarding SF1 mutation in humans remain unsolved. First, it is not known if any "milder form" of 46,XY patients with DSD, and seemingly normal adrenal function, would eventually develop late-onset adrenal insufficiency. Thus, longitudinal follow-up of these patients is mandatory. Second, if "milder form" 46,XY patients with DSD persist with normal adrenal function even on long-term follow-up, why does the SF1 mutation cause testicular dysgenesis or impaired androgen production, but not adrenal insufficiency? Third, the full phenotypic spectrum of 46,XY SF1 mutation has not been documented. Previous publications have shown that most of the patients had 46,XY DSD without adrenal insufficiency. Additionally, one patient with SF1 mutation was described to have bilateral anorchia and micropenis.¹⁷ He had a mild partial loss of SF1

function. Thus, 46,XY SF1 mutation may result in a wide spectrum of male reproductive phenotypes. Fourth, the molecular mechanism of sexual dimorphism of the SF1 phenotypes regarding gonads is not clear. The 46,XY patients showed testicular dysgenesis or impaired androgen production, whereas 46,XX patients showed an apparent normal ovarian development and function. SF1 might have different target gene(s) depending on developing fetal testis or ovary. Fifth, the molecular mechanism of the development of obesity in SF1 needs further clarification, although mice studies suggested the loss of VMH function.

SUMMARY

The identified patients with SF1 mutation definitively showed a critical role of SF1 function *in vivo*. There is a different functional importance of Sf1 (SF1) between mice and humans. In summary, in humans: (1) the important role of SF1 gene dosage has been elucidated; (2) a number of "milder form" of 46,XY patients have been reported with DSD and normal adrenal function; (3) 46,XX patients had seemingly normal ovarian development and function; and (4) adult patients might develop obesity.

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References

- Lala DS, Rice DA, Parker KL. *Mol Endocrinol*. 1992;6:1249-58.
- Parker KL, Schimmer BP. *Endocr Rev*. 1997;18:361-77.
- de-Souza B, Lin L, Achermann JC. *Pediatr Endocrinol Rev*. 2006;3:359-64.
- Luo X, Ikeda Y, Parker KL. *Cell*. 1994;77:481-90.
- Jeyasuria P, Ikeda Y, Jamin SP, et al. *Mol Endocrinol*. 2004;18:1610-9.
- Zhao L, Bakke M, Krimkevich Y, et al. *Development*. 2001;128:147-54.
- Zhao L, Ikeda Y, Stalling NR, et al. The Endocrine Society's 87th Annual Meeting, San Diego, June 4-7, 2005.
- Achermann JC, Ito M, Ito M, et al. *Nat Genet*. 1999;22:125-6.
- Achermann JC, Ozisik G, Ito M, et al. *J Clin Endocrinol Metab*. 2002;87:1829-33.
- Correa RV, Domenice S, Bingham NC, et al. *J Clin Endocrinol Metab*. 2003;89:1767-72.
- Mallet D, Bretones P, Michel-Calemard L, et al. *J Clin Endocrinol Metab*. 2004;89:4829-32.
- Hasegawa T, Fukami M, Sato N, et al. *J Clin Endocrinol Metab*. 2004;89:5930-5.
- Lin L, Philibert P, Ferraz-de-Souza B, et al. *J Clin Endocrinol Metab*. 2007;92:991-9.
- Katsumata N, Horikawa R, Ogata T, Tanaka T. In 88th Annual Meeting of the Endocrine Society, Boston USA p87, 2006.
- Reuter AL, Goji K, Bingham NC, et al. *Eur J Endocrinol*. 2007;157:233-8.
- Kohler B, Lin L, Ferraz-de-Souza B, et al. *Hum Mut*. 2008;29:59-64.
- Philibert P, Zenaty D, Lin L, et al. *Hum Reprod*. 2007;22:3255-61.
- Biason-Lauber A, Scoenle EJ. *Am J Hum Genet*. 2000;67:1563-8.
- Bland ML, Jamieson CA, Akana SF, et al. *Pro Natl Acad Sci USA*. 2000;97:14488-93.
- Majdic G, Young M, Gomez-Sanchez E, et al. *Endocrinology*. 2002;143:607-14.