A Novel Heterozygous Mutation of Steroidogenic Factor-1 (SF-1/Ad4BP) Gene (NR5A1) in a 46, XY Disorders of Sex Development (DSD) Patient without Adrenal Failure

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Abstract. Steroidogenic factor-1 [(SF-1/Ad4BP) (MIM184757)] is a nuclear receptor that regulates multiple genes involved in adrenal and gonadal development, steroidogenesis, reproduction, and other metabolic functions. Initially, mutations of SF-1/Ad4BP gene (NR5A1) in humans were identified in two 46, XY female patients with adrenal insufficiency and gonadal dysgenesis. However, recent studies have revealed that heterozygous mutations are more frequently found in 46, XY disorders of sex development (DSD) patients without adrenal failure than in 46, XY DSD patients with adrenal failure. We encountered a Japanese female patient of 46, XY DSD without adrenal failure and identified a novel mutation (V41G) of NR5A1. Functional analysis revealed that this mutant protein could not activate CYP19 promoter, indicating loss of function. In conclusion, we add a novel mutation of NR5A1 in 46, XY DSD patient without adrenal failure.

Key words: SF-1/Ad4BP, NR5A1, 46 XY DSD, Adrenal failure

(Endocrine Journal 56: 619-624, 2009)
preserved in ovary-specific Sf-1 knockout mice compared to testes in testes-specific Sf-1 knockout mice [19]. These findings indicate that only one intact SF-1/Ad4BP is sufficient for normal ovarian development.

Here, we experienced a Japanese 46, XY DSD patient and found a novel mutation (V41G) of NR5A1. In vitro study demonstrated this mutation lost its function.

Methods

DNA amplification and sequence analysis

Informed consent to participate in the study was obtained from the patient and parents. The ethical committee of Hokkaido University School of Medicine approved this study. Genomic DNA was extracted from peripheral leukocytes and each exon of NR5A1 was amplified by polymerase-chain-reaction (PCR) according to a previous report [10]. After amplification, the PCR products were purified and sequenced directly using an ABI PRISM Dye Terminator Cycle Sequencing Kit and an ABI 373A automated fluorescent sequencer.

Mutant SF-1 cDNA construction and plasmid construction

Human SF-1/Ad4BP cDNA was inserted into pcDNA 3.1 (WT-SF-1/Ad4BP). The mutant cDNA was created by site-directed mutagenesis using an overlapping PCR strategy and was designated mT-SF-1/Ad4BP. The mutation was verified by direct DNA sequencing. The human cytochrome P450arom gene (CYP19) promoter luciferase plasmid was used for analysis of SF-1/Ad4BP function as described previously [20, 21]. This construct was designated pGL3-CYP19.

Cell culture

COS cells were obtained from American Type Cell Culture and grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum.

Transient gene expression

In order to assay CYP19 promoter activity, COS cells were plated in 6-well plates, grown to 70% confluence and transiently transfected by lipofectamine with either (1) empty expression vector (pCDNA3, 0.2 µg); (2) WT-SF-1/Ad4BP (0.2 µg); (3) mT-SF-1/Ad4BP (0.2 µg); (4) WT-SF-1/Ad4BP (0.2 µg) plus mT-SF-1/Ad4BP (0.2 µg); (5) WT-SF-1/Ad4BP (0.2 µg) plus mT-SF-1/Ad4BP (0.4 µg); or (6) WT-SF-1/Ad4BP (0.2 µg) plus MT-SF-1/Ad4BP (1.0 µg) together with pGL3-CYP19 (0.4 µg). Cell extracts were prepared 48 hours after transfection and luciferase assays were performed. Luciferase measurements were divided by the respective β-galactosidase activity to control for transfection efficiency. The mean of each triplicate reaction was expressed as a percentage of the empty vector control to allow comparison of data from different experiments. Data are presented as means±S.D.

A report of case

A Japanese patient was born after 40 weeks of gestation by normal vaginal delivery and was the first child of nonconsanguineous parents. She had no siblings, and her parents were healthy. Her birth weight was 3490 g and length was 49.5 cm. At birth, clitoromegaly was noticed; however, further medical examination was not performed. Thus, the patient was reared as a female. At 12 years of age, she was referred to our hospital because of no development of secondary sexual characteristics and clitoromegaly. Her height was143 cm and body weight was 35 kg. Breast development was at Tanner stage I, and pubic hair development at Tanner stage I. She had clitoromegaly (~2.2 cm), but no posterior labial fusion and the vaginal and the urethral orifices were separated. Presumed gonads were palpable bilaterally in the in-
guinal region. Skin pigmentation was not observed. She had no episode of adrenal insufficiency during her life. Her karyotype was 46, XY. Her endocrinological evaluation is summarized in Table 1. Her serum estradiol concentration was less than 10 pg/ml. Basal serum testosterone concentration was 0.78 ng/ml and after human chorionic-gonadotropin (hCG) stimulation (5000 IU intramuscularly daily for 3 days), serum testosterone increased up to 2.57 ng/ml. Basal gonadotropin levels were elevated [follicle stimulating hormone (FSH) 41.1 mIU/ml, luteinizing hormone (LH) LH 14.35 mIU/ml].

Her basal cortisol and adrenocorticotropic (ACTH) levels were within normal range (14.8 µg/dl and 58.5 pg/ml, respectively). After ACTH stimulation, serum cortisol increased up to 20.1 µg/dl without any abnormal accumulation of adrenal steroid precursors. Pelvic magnetic resonance imaging demonstrated no uterus or vaginal pouch. Laparoscopy did not show any Mullerian derivatives. Genitoplasty and gonadectomy were performed. Histological examination revealed dysgenetic testis. Microscopic examination showed that seminiferous contained Sertoli cells, but rare germ cells, and loose interstitium had a few clusters of Leydig cells.

She is now 19 years-old and being treated with estrogen supplementation. Her height is 161 cm and body weight is 42 kg. Her basal cortisol and ACTH at 9:00 A.M. are 7.5 µg/dl and 27.5 pg/ml. Until this time, she has not developed adrenal failure.

**Results**

Sequencing analysis of NR5A1 revealed a heterozygous point mutation in exon 3 at codon 41 [GTG (Val) to GGG (Gly)] (Fig. 1A). Fifty normal Japanese subjects did not show this base change. This mutation is present in the first zinc finger domain (Fig. 1B) and this valine at residue 41 is well conserved in different species (Fig. 1C). Her parents were not subjected to DNA analysis.

In vitro transfection study demonstrated that WT-SF-1/Ad4BP activated the CYP19 promoter activity, whereas MT-SF-1/Ad4BP did not (Fig. 2). Cotransfection of mutant with WT-SF-1/Ad4BP did
not show a dominant negative effect even when 5:1 ratios of MT:WT-SF-1/Ad4BP were transfected (Fig. 2).

**Discussion**

We identified a novel mutation of V41G in a 46, XY DSD patient without adrenal insufficiency. *In vitro* promoter assay demonstrated that V41G protein lost activating function. So far C33S and G35E mutations were identified in the P-box region of the first zinc finger domain, and these two mutants lost a DNA-binding activity [6, 17]. As shown in Figure 1, the V41 residue is located near the P-box region in the first zinc finger domain, and is highly conserved among different species. Therefore, although we did not analyze DNA binding, our mutant protein would affect DNA binding, resulting in loss of function.

Despite ambiguous genitalia, our patient showed low but increased serum testosterone level after hCG stimulation at 12 years of age. These findings might be explained by insufficient testosterone production, especially during the development of the external genitalia. Most patients with SF-1/Ad4BP mutation demonstrated severe defect in testosterone production. However, low but detectable testosterone levels basal or after hCG stimulation were observed in five patients [9, 12, 14, 15, 17]. Among these patients, one had very mild phenotype of penoscrotal hypospadias and was raised as a male [15]. Consistent with the mild phenotype, the mutation (L437Q) of this patient retained partial function *in vitro*. The authors suggest the genotype may partly explain the mild phenotype. However, the other patient with increased testosterone (2.5 ng/ml) level after hCG stimulation demonstrated significant undervirilization [17]. This patient had C33S mutation with complete loss of function. Furthermore, Coutant et al. [14] have reported two siblings caused by a severe SF-1/Ad4BP mutation (c.536delC), who lack the ligand-binding domain and the activation function 2 domain. The elder 46, XY female showed ambiguous genitalia and elevated testosterone (2.2 ng/ml) level in neonatal period, which led to a presumable diagnosis of partial androgen insensitivity syndrome. By contrast, the second child of 46, XY female had less virilized genitalia than the older child and her testosterone production was severely defect. This report indicated that the difference of the phenotypes and Leydig cell function existed even in the familial case. Accordingly, not only the genotype of SF-1/Ad4BP, but also other genetic or environmental factors seem to affect testosterone production of Leydig cell during the critical period of the development of the male external genitalia. This must be further studied.

So far, 16 of the 46, XY DSD patients without adrenal failure caused by SF-1/Ad4BP mutations were reported (9-17). Lin et al. [15] have reported four 46, XY DSD patients with SF-1/Ad4BP with CYP19 promoter demonstrated the luciferase reporter gene relative to the empty vector, MT-SF-1/Ad4BP did not. Transfection of increasing amounts of MT-SF-1/Ad4BP did not impair the transactivation capacity of the wild-type protein, suggesting no dominant negative effect of the mutant protein. Data are presented as means±S.D.


Acknowledgments

We thank Dr. Ken-Ichirou Morohashi, Department of Molecular Biology Faculty of Medical Sciences, Kyushu University, for providing us with the human Ad4/BP expression vector, Dr. Toshihiko Yanase, Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, for providing human CYP19 promoter vector and Dr. Tomonobu Hasegawa, Department of Pediatrics, Keio University School of Medicine, for PCR primers for NR5A1.

References

