21-Hydroxylase Deficiency Associated with Male Infertility: Report of 2 Cases with Gene Analyses

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Abstract

We treated two patients with male infertility due to 21-hydroxylase deficiency. Endocrinologic examinations disclosed low levels of LH and FSH, with elevated ACTH and 17-OH-progesterone in both. In addition, a small testicular tumor was found in Case 1, which disappeared after adrenal replacement. Suppressed gonadotropin levels caused by increased androgen seemed to underlie the sperm dysfunction in these patients. Dexamethasone and then clomiphene were administered in Case 1, and dexamethasone in Case 2. Spermatogenesis was somewhat improved in both patients and pregnancy achieved in Case 2, though spontaneous abortion later occurred.

Key words: infertility, congenital adrenal hyperplasia, 17-hydroxyprogesterone, gonadotropin, androgen

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive inherited diseases characterized by disturbed steroidogenesis, in which more than 90% of reported cases are due to 21-hydroxylase deficiency (1, 2). 21-Hydroxylase is an enzyme that catalyzes the conversion of progesterone and 17-hydroxyprogesterone into 11-deoxycorticosterone and 11-deoxycortisol, respectively, in steroidogenesis and is encoded by the CYP21A2 gene located on chromosome 6p21.3 (3, 4).

Deficiency of this enzyme due to a mutation of the CYP 21A2 gene impairs these processes, thereby leading to excessive production of androgens, including testosterone and dihydrotestosterone, as well as lack of aldosterone and cortisol. The symptoms of 21-hydroxylase deficiency are classified into three distinct clinical phenotypes; salt-wasting, simple virilizing, and non-classical forms, according to the severity of the enzyme deficiency. The non-classical form has moderate levels of functional 21-hydroxylase, thus it is the least severe among the phenotypes, while the simple virilizing form of 21-hydroxylase deficiency is characterized by

excess androgen, which causes prenatal virilization in affected girls, and postnatal virilization in both boys and girls. Herein, we report 2 male infertility patients who were diagnosed with CAH due to the simple virilizing form of 21-hydroxylase deficiency, and discuss a possible association between excessive production of androgen and sperm dysfunction. The results of mutational analysis of the CYP21A2 gene are also presented.

Case Report

Case 1

A 37-year-old man was referred to our institution for further evaluation of infertility, as well as low levels of LH and FSH. His past medical history was unremarkable, however, during childhood he had grown rapidly and experienced precocious puberty, with pubic hair evident by the age of 7. On the other hand, he was one of the shortest in his class at the age of 14 years old, when he stopped growing in height. His parents were first cousins; their marriage was consanguineous.

A physical examination showed that the patient was hir-

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Figure 1. Sonographic image of right testis in Case 1 showing a well delineated small mass, measuring 5.9×5.1×4.7 mm, which disappeared after dexamethasone treatment.

Table 1. Sequences of Primers for Direct Sequencing

Primer	Sequence (5'→3')
AF	GAC TGC CAT TTT CTC TCT GAG ACA A
3R-2	CAA CCC CTG CTT TTC TCC CCA
3F-3	TGG GGA GAA AGC AGG GGT TG
10R-2	TGT TTA CAG GGG GGA GGG GA
2R	CCA GCC TGG GCA ACA TA
3F	AAG AAG GTC AGG CCC T
4R	TTC AGG ACA AGG AGA GG
5F	AGG CCC ACC CCC AGC CC
6R-2	CGC CTC ATA GCA ATG CTG AG
7F	TGG GCC TGT TGC CAC TC
8R	TGG GCA GCT GTG GGG TG
9F-2	CGA GGG TTG GGG ATG AGT GA

sute, while external genitalia were normal. Testicular volumes were estimated to 5 mL in the right and 6 mL in the left. Body height and weight were 160 cm and 68 kg, respectively, and blood pressure was 131/91 mmHg. The results of laboratory investigations including a complete blood count and measurement of electrolyte levels were normal (Na 137 mmol/L, K 4.2 mmol/L, Cl 101 mmol/L). In addition, ALT was 62 U/L, γ -GTP 155 U/L, and uric acid 7.2 mg/dL, with triglyceride and HDL-cholesterol levels at 338 mg/dL and 47 mg/dL, respectively, while the remainder of the routine biochemical profile was essentially within the normal limits. The karyotype was normal 46XY. A semen examination revealed azoospermia.

Endocrinologic evaluation

The patient had a normal plasma cortisol level (9.4 μ g/dL; reference range, 4-18.3 μ g/dL), while the levels of plasma progesterone (24.8 ng/mL; normal, <0.88 ng/mL), 17-hydroxyprogesterone (390 ng/mL; reference range, 0.6-3.0 ng/mL), and dehydroepiandrosterone sulfate (932 μ g/dL; reference range, 106-464 μ g/dL) were markedly increased.

Plasma ACTH (517 pg/mL; reference range, 7.2-63.3 pg/mL) and aldosterone (727 pg/mL; reference range, 35.7-240 pg/mL) concentrations were moderately increased. The plasma concentrations of free testosterone and androstenedione were 20.4 pg/mL (reference range, 7.7-21.6 pg/mL) and 21 ng/mL (reference range, 1.0-3.2 ng/mL), respectively, while those of LH and FSH were $\leq$ 0.10 mIU/mL (reference range, 0.79-5.72 mIU/mL) and 0.09 mIU/mL (reference range, 2.00-8.30 mIU/mL), respectively. The 24-hour urinary excretion of cortisol was normal (25.7 μ g/day; reference range, 11.2-80.3 μ g/day), while estrone was considerably increased (121 μ g/day; normal, <15 μ g/day). An intravenous injection of 0.25 mg of ACTH failed to increase the plasma levels of cortisol and 17-hydroxyprogesterone (cortisol: 10.1 μ g/dL at baseline, 11.8 μ g/dL at 30 minutes, 11.6 μ g/dL at 60 minutes; 17-hydroxyprogesterone: 410 ng/mL at baseline, 380 ng/mL at 30 minutes, 490 ng/mL at 60 minutes). Furthermore, the plasma concentrations of ACTH and cortisol were suppressed by administrations of dexamethasone at 1 mg (ACTH, 4.2 pg/mL; cortisol, <1.0 μ g/dL).

Imaging studies

A testicular sonogram revealed a 5.9×5.1×4.7 mm mass in the right testis (Fig. 1). Computed tomography (CT) of the abdomen showed well-circumscribed low density bilateral adrenal masses measuring 22×33 mm and 12×25 mm in diameter.

Mutational analysis of 21-hydroxylase gene (CYP 21 A2)

Written informed consent for mutational analysis was obtained after receiving approval from the ethical review board of Hyogo College of Medicine regarding the protocol.

DNA for use with a commercially available PCR assay (SRL, Tokyo, Japan) was extracted from blood. Using a DNA thermal cycler (Perkin/Elmer Cetus, Norwalk, CT), nested PCR was performed to determine the DNA sequence of 21-hydroxylase. For the first assay, a set containing the sense primer for AF (5'-GAC TGC CAT TTT CTC TCT GAG ACAA-3') and anti-sense primer for 3R2 (5'-CAA CCC CTG CTT TTC TCC CCA-3'), and another containing the sense primer for 3F-3 (5'-TGG GGA GAA AGC AGG GGT TG -3') and anti-sense primer for 10R-2 (5'-TGT TTA CAG GGA GGG GGA GGG GA-3') were used. The PCR conditions were as follows: DNA was denatured at 94°C for 5 minutes, followed by 25 cycles at 94°C for 30 seconds, then annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension step of 7 minutes. For the second PCR assay, the primers shown in Table 1 were used. For cDNA produced by the former primer set in the first PCR, the sense primer for AF and anti-sense primer for 2R were used in the second assay. For cDNA produced by the latter set in the first PCR, the sense primers for 3F, 5F, 7F, and 9F-2, and anti-sense primers for 4R, 6R-2, 8R, and 10R-2 were used in the second PCR assay. The PCR conditions, except for the set of 5F and 6R-2 were as fol-

Table 2. Endocrinological Data and Sperm Condition before and during Dexamethasone Therapy (Case 1)

	Baseline	1 month	4 months	6 months	12 months
ACTH	517	141	3.6	3.6	2.7
17-OHP	390	43	1.0	0.7	2.2
Testosterone	20.4	0.47	0.72	0.44	2.07
LH	≤0.10	0.91	1.16	1.29	2.02
FSH	0.09	2.23	4.53	4.02	4.93
DHEA-S	1250	54	19	18	35
Sperm volume			1.5 mL	1.0 mL	1.7 mL
Sperm concentration	azoospermia		8×10 ⁶ /mL	14×10 ⁶ /mL	36×10 ⁶ /mL

lows: cDNA produced in the first PCR was denatured at 94 °C for 5 minutes, followed by 25 cycles at 94 °C for 30 seconds, then annealing at 60 °C for 30 seconds and extension at 72 °C for 30 minutes with a final extension step of 7 minutes. For the set of 5F and 6R-2, the same conditions described above were used, except for an annealing temperature of 65 °C.

The PCR assays were performed in 25-μl volume containing 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl₂, 0.1% Triton X-100, 200 μM each of dATP, dCTP, dGTP, dTTP, 0.1 μg of cDNA, 10 pmol of each primer, and 1.5 U of Taq polymerase in a DNA thermal cycler (Perkin Elmer Cetus).

Mutational analysis of mRNA and genomic DNA demonstrated that the patient was a homozygote with a mutation of I172N (Ile to Asn) and heterozygote with a mutation of R356W (Arg to Trp) in the maternal allele of the CYP 21A2 gene. Genomic DNA analysis demonstrated that his mother also had heterozygous mutations of I172N and R356W, while his father had a heterozygous mutation of I172N. The laboratory findings and history of precocious puberty were compatible with a diagnosis of simple virilizing 21-hydroxylase deficiency.

Clinical course

The patient was administered 0.5 mg of oral dexamethasone, after which gonadotropin levels in his blood became normalized (Table 2) and the testicular mass disappeared. One year later, clomiphene citrate was also administered. A semen examination revealed a sperm count of 37×10⁶/mL with a motility rate of 54%. However, at the time of writing, pregnancy has not been achieved.

Case 2

A 37-year-old man visited the Department of Urology due to infertility and was referred to our department for further examinations, because of low levels of LH and FSH. His past medical and family histories were unremarkable, though the patient had visited a local physician because of pre-

cocious puberty at the age of 7. A physical examination showed that his skin was generally pigmented, while normal pubic hair distribution was noted. He had soft and small testicles (4 mL volume each) without palpable nodules. Body height and weight were 163 cm and 80 kg, respectively, and blood pressure was 160/110 mmHg. The results of laboratory investigations including a complete blood count and measurement of electrolyte levels were normal (Na 137 mmol/L, K 4.1 mmol/L, Cl 101 mmol/L). In addition, AST was 63 U/L, ALT 83 U/L, γ-GTP 156 U/L, and uric acid 7.0 mg/dL, while the remainder of the routine biochemical profile was essentially within normal limits. A semen examination revealed azoospermia.

Endocrinologic evaluation

The patient had a normal plasma cortisol level (12.2 μg/dL), while the levels of 17-hydroxyprogesterone (410 ng/mL) and dehydroepiandrosterone sulfate (869 μg/mL) in plasma were markedly increased, and those of ACTH (366 pg/mL) and aldosterone (258 pg/mL) in plasma were moderately increased. The plasma concentrations of free testosterone and androstenedione were 11.8 pg/mL and 31 ng/mL, respectively, while those of LH and FSH were <0.10 mIU/mL and 0.23 mIU/mL, respectively. The 24-hour urinary excretion of cortisol was normal (25.7 μg/day), while estrone was slightly increased (35.2 μg/day). An ACTH stimulation test (intravenous injection of 0.25 mg) yielded no response of cortisol in plasma (9.1 μg/dL at baseline, 8.2 μg/dL at 30 minutes, 9.7 μg/dL at 60 minutes).

Imaging studies

Testicular sonography revealed no tumor in either testis. Ultrasonography of the abdomen demonstrated well-circumscribed hypoechoic masses that measured 19 mm in diameter, which were located adjacent to the superior border of both kidneys (data not shown). Computed tomography (CT) and magnetic resonance imaging MRI findings suggested bilateral adrenal hyperplasia.

Table 3. Endocrinological Data and Sperm Condition before and during Dexamethasone Therapy (Case 2)

	Baseline	1 month	4 months	6 months	12 months
ACTH	366	≤2.0	≤2.0	≤2.0	≤2.0
17-OHP	410	2.5	2.6	2.3	1.8
Testosterone	6.21	2.38	2.69	2.30	1.51
LH	≤0.10	3.07	3.14	2.11	4.71
FSH	0.23	11.93	9.24	10.89	10.86
DHEA-S	869	47	14	17	14
Sperm volume			5.0 mL	3.0 mL	
Sperm concentration	azoospermia		19×10 ⁶ /mL	12×10 ⁶ /mL	

Mutational analysis of 21-hydroxylase gene (CYP 21A2)

Written informed consent for mutational analysis was obtained after receiving approval from the ethical review board of our institution regarding the protocol. Mutational analysis of mRNA and genomic DNA demonstrated that the patient was a homozygote with mutations of I172N (Ile to Asn) in a paternal allele and I172N (Ile to Asn) in a maternal allele of the CYP 21A2 gene. In addition, the results demonstrated that his father and mother also carried a heterozygous mutation of I172N. Together, these results led to our diagnosis of male infertility due to adrenal simple virilizing 21-hydroxylase deficiency.

Clinical course

Oral dexamethasone was started. Thereafter, LH and FSH concentrations in blood were normalized, and azoospermia was improved (Table 3). Pregnancy was achieved, though spontaneous abortion later occurred.

Discussion

CAH is estimated to occur in approximately 1 in 10,000 to 15,000 births worldwide (5), among which 21-hydroxylase deficiency comprises more than 90%. There are 3 different types of 21-hydroxylase deficiency, based on the clinical manifestations, two of which are classical forms, known as the salt-wasting and simple virilizing types. The third type is known as the non-classical form (6), and both males and females with this type can display signs and symptoms of excess androgen after birth. However, a diagnosis of 21-hydroxylase deficiency in male subjects who do not have symptoms of salt-wasting can be difficult. In Japan, neonatal mass screening for this autosomal recessive inheritance disease has been conducted since 1989, which has virtually eliminated undiagnosed cases of 21-hydroxylase deficiency (7). However, the present patients were both born in 1971 and had no symptoms associated with 21-

hydroxylase deficiency, except for precocious puberty in childhood, thus had not been diagnosed or treated.

The 21-hydroxylase enzyme is encoded by the CYP21A2 gene and more than 100 different mutations of CYP21A2 have been identified (8). Mutational analysis for the 21-hydroxylase gene from affected patients has demonstrated an association between enzymatic impairment and clinical manifestations (9), and genotype-phenotype relationships have been documented (10). In the present cases, genetic analysis of the CYP21A2 gene revealed mutations of I172N (Ile to Asn) in exon 4 and R356W (Arg to Trp) in exon 8 in Case 1, as well as I172N (Ile to Asn) in exon 4 in Case 2. Non-classical 21-hydroxylase deficiency may cause precocious puberty, advanced bone age, and accelerated growth velocity (11). However, typical mutations in patients with the non-classical form of 21-hydroxylase deficiency are considered to be situated in exon 7 or 1 (12). Therefore, the present patients were diagnosed as having the simple virilizing type of 21-hydroxylase deficiency, as they demonstrated precocious puberty and accelerated growth along with the I172N mutation of the CYP21A2 gene, which is associated with the simple virilizing form (13).

More than 90% of male infertility cases are associated with sperm abnormalities, such as oligospermia, asthenospermia, and teratospermia, while the remaining 10% are caused by anatomical problems, hormonal imbalances, and genetic abnormalities. Although controversial (14, 15), male infertility has been described in patients with 21-hydroxylase deficiency. Regarding the underlying mechanism of male infertility in 21-hydroxylase deficient patients, suppression of gonadotropin secretion by increased adrenal steroids due to the untreated deficiency is considered to be the cause of spermatogenesis failure (16), while it has also been suggested that the main cause of infertility in such patients is the presence of testicular adrenal rest tumors, thought to originate from aberrant adrenal tissue (17), which compress the seminiferous tubules, causing obstructive azoospermia (18). In the present two cases, though low serum concentrations of LH and FSH were observed, neither steno-

sis nor compression of seminiferous tubules by a testicular tumor was identified, which supports the speculation that the suppression of gonadotropin by increased adrenal androgens due to untreated 21-hydroxylase deficiency may result in sperm dysfunction (19).

Although it may be difficult to diagnose simple virilizing and non-classical 21-hydroxylase deficiency in adults from clinical signs, this condition should be included in the differential diagnosis for male infertility and a diagnostic approach should be performed to reveal latent 21-hydroxylase deficiency in suspected patients. Although a 17-OHP concentration in serum of greater than 6 nmol/L, which is compatible with a diagnosis of non-classical 21-hydroxylase deficiency, was detected in 11 of 222 patients (5.0%) with abnormal findings in sperm analysis conducted in a previous study, routine measurement of 17-OHP for evaluation of male infertility is not recommended, as that study found no correlations between sperm parameters and serum 17-OHP levels (20).

As for treatment, the importance of adequate glucocorticoid therapy has been emphasized to achieve successful treatment for male infertility (6). It also has been reported that clomiphene citrate treatment in addition to hormonal manipulation was successful in azoospermic patients with adrenal rests in the testes (7). Therefore, we think that it is important to optimize glucocorticoid replacement therapy to achieve normal sperm function and pregnancy.

Conclusion

Although rare, it is important to recognize 21-hydroxylase deficiency as a cause of male infertility. Furthermore, an optimal steroid dose may be required in some cases to improve infertility.

The authors state that they have no Conflict of Interest (COI).

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