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NOTE

21-Hydroxylase Deficiency Presenting as Massive Bilateral Adrenal Masses in the Seventh Decade of Life

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Abstract. A 72-year-old woman was found to have massive bilateral adrenal masses on computed tomography and was diagnosed with 21-hydroxylase deficiency (21-OHD) based on endocrinological findings. Physical examination revealed no abnormalities except markedly short stature. She was diagnosed with 21-OHD because she had an elevated serum 17α-hydroxyprogesterone (17-OHP) level which significantly decreased in response to dexamethasone. Percutaneous CT-guided biopsy and later autopsy confirmed that the adrenal masses were due to adrenocortical hyperplasia. Analysis of the *CYP21* gene revealed that the patient was a compound heterozygote for the Ile-172→Asn mutation in exon 4 and the 8-bp deletion in exon 3. Simple virilizing 21-OHD (SV) would be predicted from this genotype. She had few symptoms associated with 21-OHD except for markedly short stature, but the serum 17-OHP level was higher than that of typical nonclassical form of 21-OHD and near to that of typical SV. This finding was confirmed by analysis of the *CYP21* gene. From these results, we report that when adrenal masses are incidentally detected, 21-OHD should be ruled out to avoid excessive examination and surgery on the suspicion of adrenal carcinoma.

Key words: Bilateral adrenal masses, 21-Hydroxylase deficiency, *CYP21* gene

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21-HYDROXYLASE deficiency (21-OHD) is among the most common inherited disorders and accounts for 90–95% of congenital adrenal hyperplasia (CAH) cases [1, 2]. The two major classic disease phenotypes of 21-OHD are the salt wasting (SW) and simple virilizing (SV) forms. Nonclassical 21-OHD (NC), a milder form of the disease, is distinguished from the classical 21-OHD. Most untreated CAH patients including 21-OHD patients have adrenal masses [3, 4]. Recently, several cases of adrenal masses have been discovered incidentally on computed tomography (CT) and further work-up showed they had 21-OHD [5, 6]. The 21-hydroxylase enzyme is encoded by the functional *CYP21* gene, which is nearly identical with *CYP21P* pseudogene. Recent studies suggest that the various phenotypes of 21-OHD correspond with particular mutations in the *CYP21* gene [7–9], whereas it has been reported that the genotype does not always predict the phenotype [9, 10]. Here we report a case of 21-OHD diagnosed when bilateral adrenal masses were discovered on CT at age 72. We also discuss the diagnosis of adrenal mass and the relationship between the 21-OHD phenotype and *CYP21* genotype.

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Case report

A 72-year-old woman was referred to the Department of Medicine, Hyogo Medical Center for Adults

in June 1994 because of fever of unknown origin. Upper abdominal CT revealed bilateral massive adrenal masses, and she was admitted to the hospital for extensive examination. She denied any history of repeated vomiting or hospitalizations as an infant or child. She was 125 cm tall at age 12, when she stopped growing. She developed breasts at age 14 and pubic hair at age 15. She began to menstruate at age 17 and her menses were irregular. She was married at age 22 but was never pregnant.

Physical examination revealed that she was 128 cm tall and weighed 37 kg. Her blood pressure was 128/70 mm Hg, pulse rate was 60/min and regular, and body temperature was 37.8°C. She had sparse head hair but normal pubic hair. Slight hyperpigmentation was found in the pubic region. Examination of the genitalia revealed no clitoromegaly, scrotalization of the labial folds, or any other abnormalities.

Baseline laboratory evaluation showed the following values: leukocyte, 8800/μl; erythrocyte, 381 × 10^4/μl; Hb, 10.4 g/dl; Hct, 31.4%; Na, 138 mEq/l; K, 4.2 mEq/l; CRP, 7.3 mg/dl. Karyotype was 46XX. HLA haplotype was A24 (9), A31 (19), B60 (40), B51 (5), Cw3, DR2, DR6. The baseline cortisol and aldosterone levels were normal. The baseline ACTH level was slightly elevated (Table 1-a). Compared with the normal circadian fluctuation of ACTH and cortisol levels, the cortisol and ACTH levels at 11 p.m. in this patient were both slightly elevated (Table 1-b). Dexamethasone completely suppressed serum cortisol and ACTH. The 17α-hydroxyprogesterone (17-OHP) level was extremely high at baseline, and increased in response to ACTH stimulation test (Table 2-a). On ACTH stimulation test, serum cortisol increased within a twofold higher level from the basal level. These findings suggested slight adrenocortical insufficiency and therefore the levels of various adrenocortical steroids were measured (Table 1-a). The patient had high levels of urinary 17-ketosteroids (17-KS), pregnanetriol, and 11-deoxy-ketogenicsteroids/11-oxy-ketogenicsteroids ratio (11-deoxy-KGS/11-oxy-KGS ratio), and all were significantly suppressed in response to dexamethasone suppression test (Table 2-b).

Upper abdominal CT and magnetic resonance imaging (MRI) showed a left adrenal gland of 53 × 18 mm in size and a right adrenal gland of 32 × 23

Table 1. (a) Endocrinological findings and (b) Circadian fluctuation of serum ACTH and cortisol

(a) Hormone				(normal range)
Serum	ACTH	(pg/ml)	70	(6.1–55)
	Progesterone	(ng/ml)	9.7	(0–5.3)
	Deoxycorticosterone	(ng/ml)	0.25	(0.03–0.33)
	Aldosterone	(pg/ml)	120	(30–160)
	17-OHP	(ng/ml)	65	(0.1–3.3)
	11-deoxycortisol	(ng/ml)	0.61	(0.11–0.60)
	Cortisol	(μg/dl)	9.7	(4.4–17.4)
	DHEA-s	(ng/ml)	1630	(400–3500)
	Δ^4-androstendione	(ng/ml)	14	(0.6–5.0)
	testosterone	(ng/ml)	2.3	(0.1–0.9)
	estradiol	(pg/ml)	50	(1.1–61.7)
Urine	17-OHCS	(mg/day)	6.6	(2.4–5.8)
	11-deoxy-KGS	(mg/day)	67.3	(1.2–2.9)
	11-oxy-KGS	(mg/day)	69.9	(7.0–16.8)
	pregnanetriol	(mg/day)	34.4	(0.02–0.83)
	17-KS	(mg/day)	47.0	(3.3–13.8)

(b) Circadian fluctuation of serum ACTH and cortisol

		8 a.m.	4 p.m.	11 p.m.
ACTH	(pg/ml)	70	31	47
Cortisol	(μg/dl)	10	6.3	11

Table 2. Results of (a) ACTH stimulation test and (b) Dexamethasone supression test

(a) ACTH stimulation test*				
	(min)	0	30	60
Corsitol	(μg/dl)	9.7	14	14
17-OHP	(ng/ml)	65	—	>200

(b) Dexamethasone suppression test※						
	(day)	0	1	2	3	4
Serum						
ACTH	(pg/ml)	47	<5.0	<5.0	<5.0	<5.0
Cortisol	(μg/dl)	8.3	<1.0	<1.0	<1.0	<1.0
17-OHP	(ng/ml)	110	—	2.7	—	—
Urine						
Pregnanetriol	(mg/day)	27.2	—	—	—	<1.0
17-KS	(mg/day)	46.1	25.6	9.8	4.6	4.5
11-deoxy-KGS/11-oxy-KGS		1.15	0.88	0.86	0.61	—

*A single intravenous injection of Tetracosactide 0.25 mg
※Oral administration of Dexamethasone 2 mg/day for 2 days, followed by oral administration of 8 mg/day for another 2 days

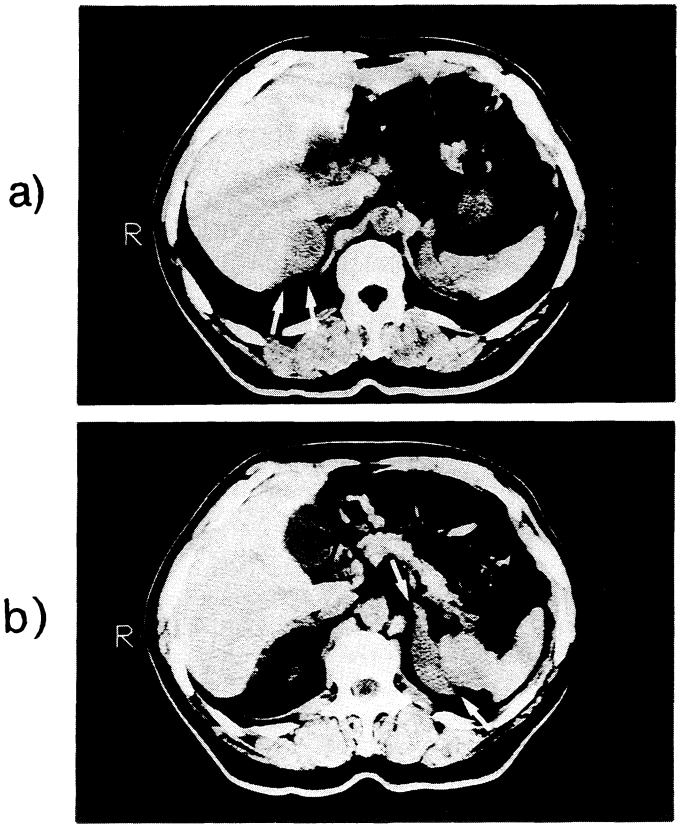

Fig. 1. CT scans of (a) the right adrenal gland and (b) the left adrenal gland. The arrows indicate the adrenal masses.

mm in size (Fig. 1). Percutaneous CT-guided biopsy of the left adrenal gland revealed no malignancy. Adrenal ^{131}I-Adosterol scintigraphy showed increased uptake by the bilateral adrenal glands. Angiography showed relatively regular tumor staining of multiple nodular states. Since the urinary 17-KS level was considerably high, blood samples were obtained from the left and right adrenal veins and two points on the inferior vena cava, that is, the central side of the turning point of the right adrenal vein and the peripheral side of the turning point of the left renal vein. The dehydroepiandrosterone-sulphate (DHEA-S), androstendione and testosterone levels in the blood samples obtained from these four points were nearly equal.

After obtaining informed consent from the patient, a genomic DNA sample was extracted from her blood leukocytes and the *CYP21* gene was analyzed. Large deletions were not detected in the *CYP21* gene by Southern blot analysis method described previously [11].

To identify point mutations, polymerase chain reaction (PCR)-restriction enzyme analysis was performed, as described previously [11]. Among the known deleterious mutations of the *CYP21* gene causing 21-OHD, we analyzed the intron 2 mutation (C or A nucleotide→G nucleotide), exon 4 mutation (Ile-172→Asn, E4IN), exon 7 mutation (Val-281 →Leu) and exon 8 mutation (Gln-318→Stop). The primer pairs which we used to amplify specific regions of the *CYP21* gene are shown in Table 3. Each of the PCR products was digested with the appropriate restriction enzyme, according to the manufacturer's recommended protocols (Boehringer Mannheim, Mannheim, Germany). After digestion, the reaction mixture was electrophoresed on a 2.5% agarose gel for 45 min at 100 mV. Only the heterozygous E4IN mutation was detected by PCR-restriction enzyme analysis (Fig. 2).

To further analyze the *CYP21* gene of the patient, DNA sequence analysis was performed according to the chain-termination method described previously [11]. For PCR followed by direct sequence analysis, the following primer pairs were used: G–H, I–J

Table 3. Sequences of primers used for PCR-restriction enzyme analysis and direct sequence analysis

Primer	Sequence	Location
A	5′-TGGGGCATCCCCAATCCAGGTCC-3′	524–546
B	5′-ACCAGCTTGTCTGCAGGAGGA ***T***-3′	677–656
C	5′-TTCTCTCTCCTCACCTGCAGCATC ***G***-3′	974–998
D	5′-CTGCATCTCCACGATGTGATCCCTC-3′	1393–1369
E	5′-GATCACATCGTGGAGATGCAGCTG-3′	1373–1396
F	5′-TGGGCCGTGTGGTGCGGTGGGGCAA-3′	2153–2129
G	5′-TCGGTGGGAGGGTACCTGAA-3′	−122–−103
H	5′-AGCAGGGAGTAGTCTCCCAAGGA-3′	722–700
I	5′-AAGGTCAGGCCCTCAGCTGCCTTCA-3′	606–630
J	5′-ATGTGCACGTGCCCTTCCAGGAG-3′	1690–1668
K	5′-AGAAGAGGGATCACATCGTGGAGAT-3′	1365–1389
L	5′-GTGGGCGCCTTGGAGGTTCGGAAT-3′	2301–2278
M	5′-TCCTTGGGAGACTACTCCCTGCT-3′	700–722

Primers A, B, C, D, E and F were used for PCR-restriction enzyme analysis. Primer pairs A–B and C–D were used for PCR-restriction enzyme analysis of the intron 2 mutation (I 2 g) and the exon 4 mutation (E4IN), respectively. In primer B and C, the T and G nucleotides at the 3′ end were mismatched to the respective nucleotides in the *CYP21* gene sequence to introduce the *Sau3AI* and *Taq*I restriction sites by PCR into the mutant sequence, respectively. Primer pair E–F was used for PCR-restriction enzyme analysis of the exon 7 mutation (E7VL) and the exon 8 mutation (E8 non). Primers G, H, I, J, K and L were used for PCR-direct sequence analysis. The primer pair M–J was used to analyze whether or not the E4IN mutation and the exon 8 deletion ($E3_8dl$) are present in the same allele. "Location" indicates the nucleotide number as described previously [12].

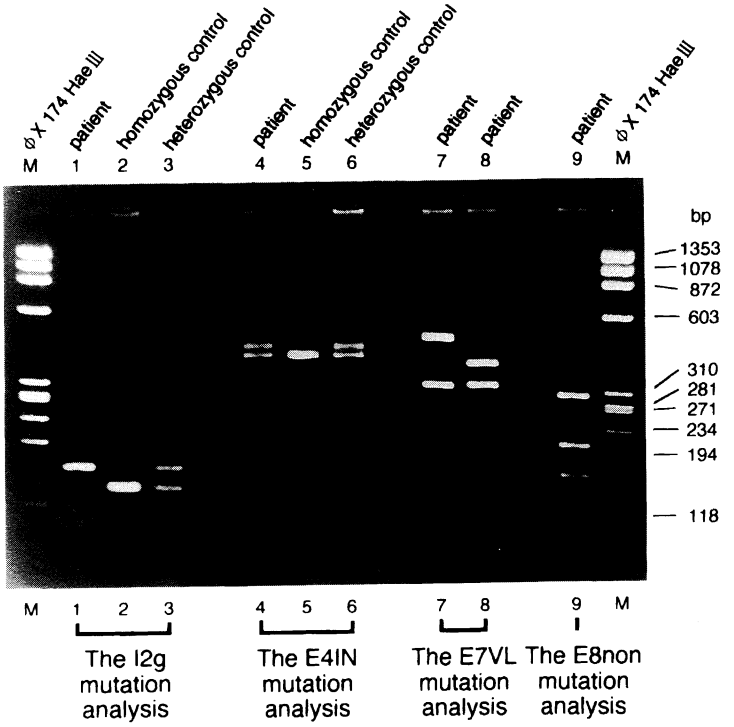

Fig. 2. Electrophoretic analysis of digested PCR products of the *CYP21* gene of the patient for detection of the intron 2 mutation (I2 g), the exon 4 mutation (E4IN), the exon 7 mutation (E7VL), and the exon 8 mutation (E8non). Lane M: molecular weight marker (ϕX174-HaeIIIdigest: Takara Shuzo Co. Ltd., Kyoto, Japan). Lanes 1–3: *Sau3A*I-digested PCR products of primer pair A-B were separated by electrophoresis for detection of the I2g mutation. The patient (lane 1) showed only a 154-bp fragment, indicating that she does not have the I2g mutation. Lanes 4–6: *Taq*I-digested PCR products of primer pair C-D were separated by electrophoresis for detection of the E4IN mutation. The patient (lane 4) showed 420-bp and 397-bp fragments, similar to the heterozygous control (lane 6), indicating that she is a heterozygote for the E4IN mutation. Lanes 7–8: *Pma*CI-digested (lane 7) and *ApaL*I-digested (lane 8) PCR products of primer pair E-F were separated by electrophoresis for detection of the E7VL mutation. Lane 7 showed 310-bp and 471-bp fragments. Lane 8 showed 375-bp, 311-bp and 95-bp fragments. These results indicate that she does not have the E7VL mutation. Lane 9: *Pst*I-digested PCR products of primer pair E-F were separated by electrophoresis for detection of the E8non mutation. The patient showed 299-bp, 204-bp, 158-bp and 120-bp fragments, indicating that she does not have the E8non mutation.

and K–L (Table 3). A heterozygous 8-nucleotide (nucleotide number 707-714 [12]) deletion in exon 3 ($E3_8dl$) was detected by DNA sequence analysis (Fig. 3).

To detect which allele carries the E4IN mutation and the $E3_8dl$ deletion, another PCR-restriction enzyme analysis was performed using primer pair M–J. Primer pair M–J was set up not to amplify the allele which carries the $E3_8dl$ deletion. The E4IN mutation was detected by this analysis, indicating that the E4IN mutation and the $E3_8dl$ deletion are present on different alleles of the *CYP21* gene.

After admission, all inflammatory findings disappeared without any treatment. Since the patient was diagnosed with 21-OHD, treatment with 0.5 mg/day oral dexamethasone was started, and the ACTH level was suppressed to less than 5.0 pg/ml. Later, the dosage of dexamethasone was decreased to 0.5 mg every two days and she was discharged. In May 1995, a metastatic liver tumor was detected on CT. Later on her tumor was confirmed as pancreatic cancer. She died in February 1996. The size of the bilateral adrenal glands on CT did not change until her death. The autopsy revealed a left adrenal gland of 29 g measuring $80 \times 60 \times 18$ mm, and a right adrenal gland of 40 g measuring $90 \times 70 \times 20$ mm. The histologic diagnosis of the bilateral masses was adrenocortical hyperplasia.

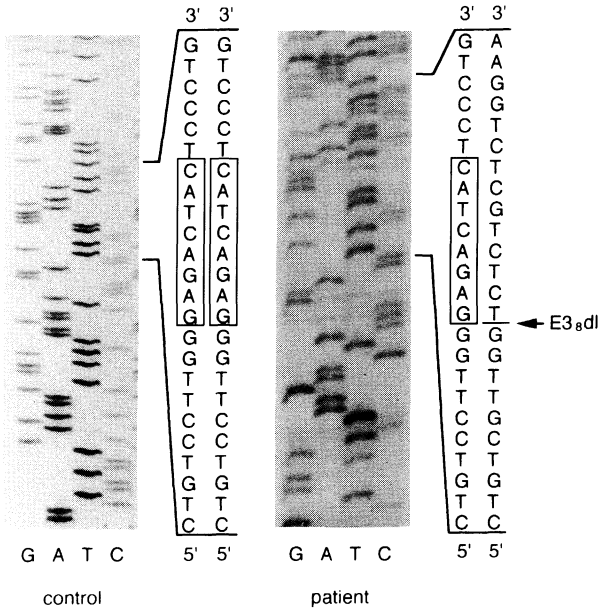

Fig. 3. Direct sequence analysis of a portion of exon 3 of the *CYP21* gene of the patient. The PCR product of primer pair I-J was subjected to PCR-direct sequence analysis. In the sequence ladder of the control, only normal sequence was present. In the sequence ladder of the patient, two different sequences were present. The sequence represented on the left in the sequence ladder is normal, while the sequence represented on the right in the sequence ladder lacks the sequence "GAGACTAC" (nucleotide number 707 to 714 [12]). This finding indicates that the patient is a heterozygote for the 8-nucleotide deletion in exon 3 ($E3_8dl$).

Discussion

Our patient was diagnosed with 21-OHD because she had elevated levels of serum 17-OHP, urinary pregnanetriol, and urinary 11-deoxy-KGS/11-oxy-KGS ratio, all of which decreased in response to dexamethasone. She denied any history of salt-wasting episodes and had few symptoms except for markedly short stature which is considered to be associated with 21-OHD. The clinical diagnosis is commonly confirmed by analysis of the *CYP21* gene [7–9]. Our patient was a compound heterozygote for the E4IN mutation and the $E3_8dl$ deletion. The $E3_8dl$ deletion renders the gene nonfunctional by generating premature termination [8], and this deletion is often detected in the *CYP21* gene of SW patients [13]. The E4IN mutation reduces 21-hydroxylase enzyme activity to less than 10% of normal activity [8], and this mutation is often found in the *CYP21* gene of SV patients [9, 14]. Therefore, a compound heterozygote for these two nucleotide alterations should result in the SV phenotype, considering that 21-OHD is an autosomal recessive disease. Speiser *et al.* investigated the relationship between 17-OHP level and phenotype of 21-OHD, and reported that the basal level of serum 17-OHP in SV patients is usually >300 nmol/l (99 ng/ml), while NC patients have a lower basal level of 17-OHP [9]. In our case, the serum 17-OHP level was higher than that of typical NC and near to that of typical SV.

As most untreated CAH patients including 21-OHD patients have adrenal masses [3, 4], our patient was diagnosed as having 21-OHD when bilateral adrenal masses were incidentally discovered on CT. Jaresch *et al.* examined 22 patients with homozygous CAH (of which 20 patients had 21-OHD) who were under replacement therapy with hydrocortisone, and reported that adrenal tumors were detected in 82% of the patients on CT [4]. Twenty siblings of the 22 homozygous CAH patients were diagnosed with heterozygous CAH (of which 19 siblings had 21-OHD), and adrenal tumors were detected in 45% of the siblings with heterozygous CAH. Therefore, in order to detect NC or heterozygous CAH, patients with adrenal masses should have an ACTH stimulation test unless the cause of the mass is otherwise evident [5]. Small incidentalomas are markedly less likely to be carcinomas than large incidentalomas. Therefore, Ravichandran *et al.* mentioned that CAH patients with smaller incidentalomas should be followed both clinically and with periodic CT scans, and that biopsy or surgery need not be routine [5]. In our patient, as both adrenal masses exceeded 3 cm in diameter and the inner part of the masses showed heterogeneity, adrenocortical carcinoma could not be completely ruled out. Therefore, percutaneous CT-guided biopsy was performed. Efforts should be made to check for 21-OHD in patients with adrenal masses discovered incidentally, to avoid excessive examination and surgery.

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