Identification of a homozygous c.1039C>T (p.R347C) variant in CYP17A1 in a 67-year-old female patient with partial 17α-hydroxylase/17,20-lyase deficiency

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Abstract. 17α-Hydroxylase/17,20-lyase deficiency (17OHD) is caused by pathogenic mutations in CYP17A1. Impaired 17α-hydroxylase and 17,20-lyase activities typically induce hypertension, hypokalemia, sexual infantilism, and amenorrhea. Most patients with 17OHD are diagnosed in adolescence. Here, we report a female (46, XX) patient with 17OHD who was diagnosed at the age of 67 years. Genetic analysis was performed using direct DNA sequencing of polymerase chain reaction (PCR) products and multiplex ligation-dependent probe amplification (MLPA) analysis. Direct DNA sequencing revealed a homozygous c.1039C>T in CYP17A1, corresponding to a p.R347C amino acid change. MLPA probe signals showed that the CYP17A1 mutation was present in the homozygous carrier state. The patient’s dehydroepiandrosterone sulfate and androstenedione levels were extremely low, despite elevated adrenocorticotropic hormone (ACTH) and normal cortisol levels. A corticotropin-releasing hormone (CRH) test showed no response of cortisol, despite a normal response of ACTH. Rapid ACTH injection resulted in elevations in the deoxycorticosterone, corticosterone, aldosterone, and 17-hydroxypregnenolone levels, but not in the cortisol level. These results suggested that 17α-hydroxylase/17,20-lyase activities were partially impaired. Computed tomography revealed bilateral adrenal hyperplasia and a hypoplastic uterus. A high basal plasma ACTH level and a discrepancy between ACTH and cortisol responses in a CRH test may provide a definitive diagnostic clue for this disease.

Key words: 17α-hydroxylase/17,20-lyase deficiency, Adrenal hyperplasia
and hypokalemia (2.6 mmol/L). Plasma adrenocorticotropic hormone (ACTH) and cortisol levels at 9:00 AM were 66.6–78.7 pg/mL and 12.9–17.3 μg/dL, respectively. As ACTH-dependent Cushing’s syndrome was also suspected, a 0.5 mg dexamethasone suppression test and magnetic resonance imaging (MRI) were performed by the previous doctor. After 0.5 mg dexamethasone administration, ACTH and cortisol levels at 9:30 AM were 20 pg/mL and 5.3 μg/dL, respectively. The presence of a pituitary tumor (5 mm) was suspected on MRI. The patient was treated with oral antihypertensive agents.

On admission at our hospital, the patient’s blood pressure was 162/84 mmHg, with a regular heart rate of 76 beats per minute. The patient’s height was 155 cm, body weight was 56 kg, and body mass index was 23.3 kg/m². She had no problem concerning the progression of secondary sexual characteristics, although she was infertile. She had poor development of pubic and axillary hair. No pigmentation or Cushingoid features were noted. Endocrinological examinations revealed a high baseline level of ACTH (114 pg/mL) and a normal cortisol level (11.3 μg/dL) at 9:30 AM. A high plasma aldosterone level (30.4 ng/dL) and low plasma renin activity (0.7 ng/mL/h) were found in her data (Table 1). Plasma aldosterone levels were 17.2 (60 min) and 15.7 ng/dL (90 min) after oral administration of 50 mg captopril (Table 2). The data corresponded to the diagnostic criteria for primary aldosteronism in the guidelines of the Japan Endocrine Society [7]. Blood levels of dehydroepiandrosterone sulfate (5.0 μg/dL) and estradiol (<5.0 pg/mL) were low, whereas the progesterone level was high (3.8 ng/mL) (Table 1). Urinary free cortisol (56.8 and 86.2 μg/day) and urinary aldosterone (1.8 and 2.4 μg/day) levels were within the normal range (Table 3). Because of suspected pituitary adenoma on MRI, corticotropin-releasing hormone (CRH) test and 1 mg dexamethasone test were performed during hospitalization. The CRH test revealed no response of cortisol (from 9.0 μg/dL to 9.7 μg/L) despite a normal response of ACTH (from 53 pg/mL to 213 pg/mL) (Table 2). Three-tesla MRI showed no pituitary tumor. As adrenal insufficiency was suspected, a rapid ACTH test was performed. The rapid ACTH test (250 μg Synacthen) revealed a low peak level of cortisol (10.2 μg/dL), despite a robust elevation in aldosterone levels (from 18.1 ng/dL to 37.3 ng/dL) (Table 2). Additionally, peak levels of DOC, corticosterone, and 17α hydroxyprogrenenolone exhibited robust responses in the rapid ACTH test (1.2, 199, and 7.7 ng/mL, respectively) (Table 2). After the administration of 1 mg dexamethasone, the ACTH and cortisol levels decreased to 13.5 pg/mL and 4.8 μg/dL, respectively, in the morning. Although the urinary level of 17-ketosteroid was not detected, 17-ketogenic sterol (KGS) (6.2 and 6.5 mg/day),

### Table 1 Laboratory data

| Parameter                  | Units | Range      | Reference range |
|----------------------------|-------|------------|-----------------|
| ACTH (pg/mL)               |       | 114        | (7.2–63.3)      |
| Cortisol (μg/dL)           |       | 11.3       | (4.5–21.1)      |
| Aldosterone (ng/dL)        |       | 30.4       | (3.6–24.0)      |
| PRA (ng/mL/h)              |       | 0.7        | (0.3–2.9)       |
| DHEAS (μg/dL)              |       | 5.0        | (13.0–154.0)    |
| LH (μIU/mL)                |       | 18.7       | (1.4–15.0)      |
| FSH (μIU/mL)               |       | 53         | (0.5–5.0)       |
| Estradiol (pg/mL)          |       | <5.0       | (19.5–144.2)    |
| Progesterone (ng/mL)       |       | 3.8        | (<0.33)         |
| Pregnenolone (ng/mL)       |       | 1.2        | (0.2–1.5)       |
| 11-deoxycorticosterone (ng/mL) |   | 0.3       | (0.03–0.33)     |
| 11-deoxycortisol (ng/mL)   |       | 0.6        | (0.11–0.60)     |
| Androstenedione (ng/mL)    |       | <0.10      | (0.25–1.21)     |
| PRA, plasma renin activity; DOC, deoxycorticosterone; OHP, hydroxyprogrenenolone; DHEAS, dehydroepiandrosterone sulfate |

### Table 2 Endocrinological Examination. Captopril (50 mg) was administered orally. Human CRH (100 μg) and ACTH (250 μg Synacthen) were administered intravenously

| Test                      | Time | Units | Range        |
|---------------------------|------|-------|--------------|
| Captopril test            | 0'   |       |              |
| Aldosterone (ng/dL)       | 0'   | 21.0  | 15.7         |
| Renin activity (ng/mL/h)  | 0'   | 1.1   | 1.4          |
| CRH test                  | 0'   |       |              |
| ACTH (pg/mL)              | 53   | 168   | 213          |
| Cortisol (μg/dL)          | 9.0  | 9.0   | 9.3          |
| Rapid ACTH test           | 0'   |       |              |
| Cortisol (μg/dL)          | 9.2  | 9.7   | 10.2         |
| Aldosterone (ng/dL)       | 18.1 | 30.6  | 37.3         |
| DOC (ng/mL)               | 0.3  | 1.1   | 1.2          |
| Corticosterone (ng/mL)    | 28.4 | 164   | 199          |
| 17αOHP (ng/mL)            | 3.4  | 6.2   | 7.7          |
| DOC, deoxycorticosterone; OHP, hydroxyprogrenenolone |

### Table 3 Urinary hormones

| Parameter                    | Units | Range       | Reference range |
|------------------------------|-------|-------------|-----------------|
| 24-hour urinalysis           |       | 56.8–86.2   | (11.2–80.3)     |
| Cortisol (μg/day)            |       | 1.8–2.4     | (<10.0)         |
| Aldosterone (μg/dL)          |       | 6.2–6.5     | (3.5–11.2)      |
| 17-KGS (mg/day)              |       | 1.3–1.6     | (1.5–3.9)       |
| 11-deoxyKGS (mg/day)         |       | 3.3–4.5     | (3.3–8.1)       |
| 11-oxyKGS (mg/day)           |       | not detected| (6.0–18.4)      |
| 17-ketosteroids (mg/day)      |       | not detected| (6.0–18.4)      |
| KGS, ketogenic steroid |

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11-deoxyKGS (1.3 and 1.6 mg/day), and 11-oxyKGS (3.3 and 4.5 mg/day) levels were within the normal ranges on each day of 24-hour urine collection for 2 days (Table 3).

Karyotype analysis showed 46, XX. A computed tomography scan revealed a bilaterally enlarged adrenal and uterine hypoplasia (Fig. 1). 17OHD was diagnosed based on the results of these biochemical and imaging evaluations.

After obtaining informed consent from the patient, genomic analysis was conducted using DNA from peripheral blood leukocytes [8]. Direct DNA sequencing of polymerase chain reaction (PCR) products disclosed a homozygous c.1039C>T variant in exon 6 of CYP17A1 (p.R347C) (Fig. 2A). To exclude a heterozygous deletion of CYP17A1, multiplex ligation-dependent probe amplification (MLPA) analysis was conducted. MLPA probe signals from exons 1 to 7 were neither eliminated nor reduced, indicating that the CYP17A1 variant was present in a homozygous carrier state (Fig. 2B). For treatment of hyperaldosteronism, spironolactone (25 mg/day) was administered, and her blood pressure and hypokalemia improved 1 month later.

Fig. 1 Abdominal contrast-enhanced computed tomography (CT). The image demonstrates bilaterally enlarged adrenal glands.

Fig. 2 (A) Direct sequencing of polymerase chain reaction (PCR) products. A point mutation, c.1039C>T (p.R347C), at codon 347 in exon 6 is detected. (B) Multiplex ligation-dependent probe amplification (MLPA) probe signal for CYP17A1. The result shows a normal MLPA probe signal from exons 1–7.
The present case illustrates the need for scrutinizing an application of MLPA in the diagnosis of 17OHD [12-14].

Steroid 17α-hydroxylase and 17,20-lyase (cytochrome P450c17) are encoded by CYP17A1 [10]. CYP17A1 is localized on chromosome 10q24.3 and has 8 exons, coding for a protein of 508 amino acids [10, 11]. The c.1039C>T (p.R347C) variant of CYP17A1 has been described in two male (46, XY) patients with hypertension and sexual infantilism: one patient was a compound heterozygote for p.R347C and had a 25 bp deletion in exon 1, whereas the other patient had a 4 bp duplication in exon 8 on the other allele [9]. Gonadectomy was performed in these patients because they both had complete female external genitalia with absent uterus. After gonadectomy, the patients experienced hypertension, despite normal renin levels [9]. They were finally diagnosed as having 17OHD at the ages of 10 and 28 years, respectively [9].

Our patient was the first female (46, XX) case with p.R347C mutation in CYP17A1. Direct DNA sequencing of PCR products revealed a homozygous mutation pattern. However, as this pattern comprises both net homozygous and heterozygous deletion patterns, we next conducted MLPA analysis to rule out heterozygous deletion in codon 347. The MLPA probe signals confirmed that CYP17A1 mutation was present in a homozygous carrier state in our patient. 17OHD is inherited in an autosomal recessive manner. Therefore, parental consanguinity is an important risk factor [2]. Although the patient’s parental DNA was not available, she was a daughter of consanguineous parents. The genetic analysis results supported that her parents’ marriage was consanguineous. The MLPA method is mainly employed in the molecular diagnosis of CAH owing to 21-hydroxylase deficiency for the detection of deletions/duplications in CYP21A2. There are few studies on the application of MLPA in the diagnosis of 17OHD [12-14]. The present case illustrates the need for scrutinizing a homozygous mutation pattern by not only direct DNA sequencing but also MLPA analysis.

CYP17A1 is a key enzyme in glucocorticoid and sex hormone biosynthesis, and is mainly expressed in the adrenal cortex and gonads as a dual-functional monoxygenase facilitating both 17α-hydroxylase and 17,20-lyase activities [15]. The 17α-hydroxylase reaction converts pregnenolone to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone. These products are converted by 17,20-lyase activity to dehydroepiandrosterone and androstenedione in the adrenal glands and gonads, respectively [16]. The lack of adrenal 17α-hydroxylase activity causes hypertension and hypokalemia by driving overproduction of the 17-deoxysteroids, 11-DOC, and corticosterone. Typically, an excess of these mineralocorticoids may lead to the inhibition of renin and aldosterone production. Indeed, Yamakita et al. reported that 29% of 17OHD patients had hyperaldosteronism [17]. Yanase et al. also reported hyperaldosteronism in 17 of 122 cases with 17α-hydroxylase deficiency [2]. Autonomous aldosterone production might be suggested when the captopril test is positive. In our case, plasma aldosterone levels were high, whereas urinary aldosterone levels were within the lower normal. The rapid ACTH test revealed a robust elevation in aldosterone levels. Both 18-hydroxydeoxycorticosterone and 18-hydroxy corticosterone, the immediate precursors of aldosterone, are known to be elevated by the increase in ACTH levels in zona fasciculata in 17OHD [18, 19]. Therefore, aldosterone synthesis in the adrenal zona fasciculata is increased [17, 19], whereas aldosterone production in the zona glomerulosa might be suppressed by excess DOC/corticosterone levels [20]. Such balances in aldosterone production may differ among the cases of 17OHD. The age of the onset of hypertension, degree of hypokalemia, and aldosterone production rate vary even among patients with mutations that completely inactivate the enzyme [2, 6]. Loss of 17,20-lyase activity also precludes sex steroid synthesis and leads to sexual infantilism, delayed puberty, and primary amenorrhea in females. Although most of the patients with 17OHD are diagnosed before adolescence, some patients with retained menstruation are undiagnosed until adulthood [2, 5, 6].

In our patient, basal plasma and 24 h-urinary levels of cortisol were normal. However, a rapid ACTH test revealed an adrenal steroidogenic failure, owing to the weak responses of cortisol and the strong responses of DOC, corticosterone, aldosterone, and 17α-hydroxyprogrenolone. The elevated basal ACTH level in the blood and suppression of the plasma ACTH level by 0.5 and 1 mg dexamethasone suggested that this patient may have latent hypocortisolemia. The inadequate suppression of cortisol by 0.5 and 1 mg dexamethasone may be due to cross-reactivity with other adrenal steroid hormones because cross-reactivity of 0.64% and 2.48% was reported for DOC and corticosterone, respectively, in electrochemiluminescence immunoassay [21]. In fact, the CRH test indicated adrenal insufficiency, given the low peak cortisol level. CRH testing has been widely
used as a noninvasive tool for the differential diagnosis of ACTH-dependent Cushing’s syndrome. Most patients with Cushing’s disease show increased ACTH and cortisol levels in a CRH test [22]. In our patient, a latent adrenal insufficiency was revealed by the CRH test, as her cortisol level was not increased, despite the normal response of ACTH. Therefore, a discrepancy between ACTH and cortisol responses as indicated by the CRH test may provide a definitive diagnostic clue for this disease.

Our patient was diagnosed at the age of 67 years, and basal levels of androstenedione, estradiol, and dehydroepiandrosterone sulfate were all undetectable. Although it is unknown when her hypogonadism started, her adrenal or ovary androgen levels might have been sufficient for the development of secondary sexual characteristics and normal menstruation until menopause, as she had combined deficiencies in 17α-hydroxylase and 17,20-lyase activities. In a 46, XX individual, the occurrence of pubertal arrest or primary amenorrhea depends on enzymatic rest function, because 5%–8% of 17,20-lyase activity may suffice for pubertal development [2, 18, 23]. In previous p.R347C mutant cases of 17OHD, in vitro 17α-hydroxylase and 17,20-lyase activities of 13.6% and <1%, respectively, have been reported [9]. The p.R347C variant is present in the redox partner binding site of P450c17 [10, 24-31]. Especially, Arg-347 of CYP17A1 is critical for the cytochrome b5-dependent 17α-hydroxylase/17,20-lyase enzymatic activities by weakening the binding of P450-oxidoreductase (OR) and cytochrome b5 [10, 24-31]. Additionally, in COS-1 cells, the overexpressed cytochrome b5 selectively restored the enzyme activity and clinical severity remain unclear, homozygous p.R347C mutation may maintain sufficient 17α-hydroxylase/17,20-lyase enzyme activities to avoid adrenal insufficiency and amenorrhea.

**Conclusion**

We report the case of a patient with partial 17OHD who was first diagnosed at the age of 67 years. The patient had a genuine homozygous p.R347C variant. A high basal plasma ACTH level and a discrepancy between ACTH and cortisol responses in a CRH test may provide a definitive diagnostic clue for this disease.

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**Disclosure Statement**

None of the authors have anything to disclose.

**Conflicts of Interest**

None of the authors have potential conflicts of interest to declare.

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