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

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Abstract. 17 α -Hydroxylase/17,20-lyase deficiency (17OHD) is caused by pathogenic mutations in *CYP17A1*. Impaired 17 α -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 adrenocorticotrophic 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 17 α -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: 17 α -hydroxylase/17,20-lyase deficiency, Adrenal hyperplasia

17 α -HYDROXYLASE/17,20-LYASE DEFICIENCY (17OHD) is a rare form of congenital adrenal hyperplasia (CAH), with an estimated incidence of 1:50,000–100,000, accounting for 1% of all CAH cases [1]. 17OHD is an autosomal recessive disorder caused by homozygous or compound heterozygous variants in *CYP17A1*, which encodes P450c17, involved in glucocorticoid and sex hormone biosynthesis [2, 3]. Blockage of the pathway catalyzed by 17 α -hydroxylase stimulates ACTH in the pituitary, leading to the overproduction of 11-deoxycorticosterone (DOC) and corticosterone, resulting in hypertension and hypokalemia [4]. Hypergonadotropic hypogonadism is the clinical hallmark of 17OHD and presents as sexual infantilism and primarily, amenorrhea,

in women. Most patients with 17OHD are diagnosed in adolescence. However, mild or intermediate phenotypes of 17OHD are generally diagnosed in adulthood or at older age because of preserved gonadal function [2, 5, 6].

Here, we describe a female case of 17OHD diagnosed at the age of 67 years. To our knowledge, this is the oldest patient with 17OHD reported to date [2, 6].

Case Report

The patient, a 67-year-old woman, was referred to our hospital for further diagnosis and treatment of hypertension, hypokalemia, and suspected pituitary microadenoma. Total thyroidectomy was performed for papillary thyroid carcinoma at the age of 53 years. The patient had been hypertensive for 2 years; however, she had not visited a medical institution because she had no symptoms. Her previous doctor suspected primary aldosteronism because of a high plasma aldosterone level (16.7 ng/dL), low plasma renin activity (0.6 ng/mL/h),

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and hypokalemia (2.6 mmol/L). Plasma adrenocorticotrophic 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/dL) 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 hydroxypregnenolone 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 steroid (KGS) (6.2 and 6.5 mg/day),

Table 1 Laboratory data

		(Reference ranges)
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, hydroxypregnenolone; 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

Captopril test	0'	60'	90'	
Aldosterone (ng/dL)	21.0	17.2	15.7	
Renin activity (ng/mL/h)	1.1	1.4	1.4	
CRH test	0'	30'	60'	90'
ACTH (pg/mL)	53	168	213	144
Cortisol (µg/dL)	9.0	9.0	9.3	9.7
Rapid ACTH test	0'	30'	60'	
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, hydroxypregnenolone

Table 3 Urinary hormones

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

KGS, ketogenicsteroid

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.

Discussion

The patient had hypertension and hypokalemia accompanying hyperaldosteronism. Direct DNA sequencing of PCR products and MLPA analysis revealed a homozygous c.1039C>T in *CYP17A1*, corresponding to a p.R347C amino acid alteration. Although two male (46, XY) cases with heterogeneous p.R347C mutation have been reported [9], this is the first case of 17OHD with a 46, XX karyotype and homozygous p.R347C mutation.

Steroid 17 α -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 monooxygenase facilitating both 17 α -hydroxylase and 17,20-lyase activities [15]. The 17 α -hydroxylation reaction converts

pregnenolone to 17 α -hydroxypregnenolone and progesterone to 17 α -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 17 α -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 17 α -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-hydroxycorticosterone, 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 17 α -hydroxypregnenolone. 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 17 α -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* 17 α -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 [9]. Because the redox partner interaction domain is essential for the 17 α -hydroxylase/17,20-lyase function of P450c17, this mutation modulates the 17 α -hydroxylase/17,20-lyase enzymatic activities by weakening the binding of P450-oxidoreductase (OR) and cytochrome b5 [10, 24–31]. Especially, Arg-347 of CYP17A1 is critical for the cytochrome b5-dependent acyl-carbon cleavage activity [24, 30]. In relation to the codon 347 of CYP17A1, a homozygous c.1040G>A (p.R347H) mutation has been described [9, 24]. Unlike

p.R347C, a p.R347H mutation evokes an isolated 17,20-lyase deficiency [9, 24]. Interestingly, in COS-1 cells, overexpressed cytochrome b5 selectively restored the 17,20-lyase activity in the R347H mutant in the presence of OR [26]. Thus, we hypothesized the cortisol secretion and gonadal function in our patient might be maintained due to cytochrome b5, together with the *in vivo* accumulation of OR.

Although the relationships between *in vitro* enzyme activity and clinical severity remain unclear, homozygous p.R347C mutation may maintain sufficient 17 α -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.

References

1. New MI (2003) Inborn errors of adrenal steroidogenesis. *Mol Cell Endocrinol* 211: 75–83.
2. Yanase T, Simpson ER, Waterman MR (1991) 17 alpha-hydroxylase/17,20-lyase deficiency: from clinical investigation to molecular definition. *Endocr Rev* 12: 91–108.
3. Auchus RJ (2017) Steroid 17-hydroxylase and 17,20-lyase deficiencies, genetic and pharmacologic. *J Steroid Biochem Mol Biol* 165: 71–78.
4. Biglieri EG, Kater CE (1991) 17 alpha-hydroxylation deficiency. *Endocrinol Metab Clin North Am* 20: 257–268.
5. Katayama Y, Kado S, Wada S, Nemoto Y, Kugai N, *et al.* (1994) A case of 17 α -hydroxylase deficiency with retained menstruation. *Endocr J* 41: 213–218.
6. Ueda Y, Usui T, Watanabe T, Kaneko K, Nakatani R, *et al.* (2015) Elevated levels of plasma immunoassayable aldosterone in a mild form of 17 alpha-hydroxylase/17,20-lyase deficiency diagnosed at the age of 50. *AACE Clin Case Rep* 1: e156–e160.
7. Nishikawa T, Omura M, Satoh F, Shibata H, Takahashi K, *et al.* (2011) Guidelines for the diagnosis and treatment of primary aldosteronism—the Japan Endocrine Society 2009. *Endocr J* 58: 711–721.
8. Usui T, Nishisho K, Kaji M, Ikuno N, Yorifuji T, *et al.* (2004) Three novel mutations in Japanese patients with 21-hydroxylase deficiency. *Horm Res* 61: 126–132.
9. Van Den Akker EL, Koper JW, Boehmer AL, Themmen AP, Verhoef-Post M, *et al.* (2002) Differential inhibition of 17 α -hydroxylase and 17,20-lyase activities by three

- novel missense CYP17 mutations identified in patients with P450c17 deficiency. *J Clin Endocrinol Metab* 87: 5714–5721.
10. Picado-Leonard J, Miller WL (1987) Cloning and sequence of the human gene for P450c17 (steroid 17 α -hydroxylase/17,20 lyase): similarity with the gene for P450c21. *DNA* 6: 439–448.
 11. Sparkes RS, Klisak I, Miller WL (1991) Regional mapping of genes encoding human steroidogenic enzymes: P450scc to 15q23-q24, adrenodoxin to 11q22; adrenodoxin reductase to 17q24-q25; and P450c17 to 10q24-q25. *DNA Cell Biol* 10: 359–365.
 12. Turkkahraman D, Guran T, Ivison H, Griffin A, Vijzelaar R, *et al.* (2015) Identification of a novel large CYP17A1 deletion by MLPA analysis in a family with classic 17 α -hydroxylase deficiency. *Sex Dev* 9: 91–97.
 13. Bolu S, Eröz R, Tekin M, Doğan M (2020) Atypical presentation in patients with 17 α -hydroxylase deficiency caused by a deletion in the CYP17A1 gene: short stature. *Turk J Pediatr* 62: 851–857.
 14. Kardelen AD, Toksoy G, Baş F, Yavaş Abalı Z, Gençay G, *et al.* (2018) A rare cause of congenital adrenal hyperplasia: clinical and genetic findings and follow-up characteristics of six patients with 17-hydroxylase deficiency including two novel mutations. *J Clin Res Pediatr Endocrinol* 10: 206–215.
 15. Nakajin S, Shinoda M, Haniu M, Shively JE, Hall PF (1984) C21 steroid side chain cleavage enzyme from porcine adrenal microsomes. Purification and characterization of the 17 α -hydroxylase/C17,20-lyase cytochrome P-450. *J Biol Chem* 259: 3971–3976.
 16. Auchus RJ (2001) The genetics, pathophysiology, and management of human deficiencies of P450c17. *Endocrinol Metab Clin North Am* 30: 101–119, vii.
 17. Yamakita N, Murase H, Yasuda K, Noritake N, Mercado-Asis LB, *et al.* (1989) Possible hyperaldosteronism and discrepancy in enzyme activity deficiency in adrenal and gonadal glands in Japanese patients with 17 α -hydroxylase deficiency. *Endocrinol Jpn* 36: 515–536.
 18. Costa-Santos, M, Kater CE, Auchus RJ, Brazilian Congenital Adrenal Hyperplasia Multicenter Study Group (2004) Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. *J Clin Endocrinol Metab* 89: 49–60.
 19. Kater CE, Biglieri EG (1994) Disorders of steroid 17 α -hydroxylase deficiency. *Endocrinol Metab Clin North Am* 23: 341–357.
 20. Bassett MH, White PC, Rainey WE (2004) The regulation of aldosterone synthase expression. *Mol Cell Endocrinol* 217: 67–74.
 21. Method-sheet-cobas [Internet]. Roche Diagnostics GmbH, Mannheim. Available from: http://labogids.sintmaria.be/sites/default/files/files/cortisol_ii_2017-05_v4.pdf accessed on June 24, 2021
 22. Newell-Price J, Morris DG, Drake WM, Korbonits M, Monson JP, *et al.* (2002) Optimal response criteria for the human CRH test in the differential diagnosis of ACTH-dependent Cushing's syndrome. *J Clin Endocrinol Metab* 87: 1640–1645.
 23. Miura K, Yasuda K, Yanase T, Yamakita N, Sasano H, *et al.* (1996) Mutation of cytochrome P-45017 α gene (CYP17) in a Japanese patient previously reported as having glucocorticoid-responsive hyperaldosteronism: with a review of Japanese patients with mutations of CYP17. *J Clin Endocrinol Metab* 81: 3797–3801.
 24. Geller DH, Auchus RJ, Mendonça BB, Miller WL (1997) The genetic and functional basis of isolated 17,20 lyase deficiency. *Nat Genet* 17: 201–205.
 25. Auchus RJ, Miller WL (1999) Molecular modeling of human P450c17 (17 α -hydroxylase/17,20-lyase): insights into reaction mechanisms and effects of mutations. *Mol Endocrinol* 13: 1169–1182.
 26. Geller DH, Auchus RJ, Miller WL (1999) P450c17 mutations R347H and R358Q selectively disrupt 17,20-lyase activity by disrupting interactions with P450 oxidoreductase and cytochrome b5. *Mol Endocrinol* 13: 167–175.
 27. Miller WL (2005) Minireview: regulation of steroidogenesis by electron transfer. *Endocrinology* 146: 2544–2550.
 28. Naffin-Olivos JL, Auchus RJ (2006) Human cytochrome b5 requires residues E48 and E49 to stimulate the 17, 20-lyase activity of cytochrome P450c17. *Biochemistry* 45: 755–762.
 29. Peng HM, Liu J, Forsberg SE, Tran HT, Anderson SM, *et al.* (2014) Catalytically relevant electrostatic interactions of cytochrome P450c17 (CYP17A1) and cytochrome b5. *J Biol Chem* 289: 33838–33849.
 30. Lee-Robichaud P, Akhtar ME, Wright JN, Sheikh QI, Akhtar M (2004) The cationic charges on Arg347, Arg358, and Arg449 of human cytochrome P450c17 (CYP17) are essential for the enzyme's cytochrome b5-dependent acyl-carbon cleavage activities. *J Steroid Biochem Mol Biol* 92: 119–130.
 31. Auchus RJ, Lee TC, Miller WL (1998) Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *J Biol Chem* 273: 3158–3165.