0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(3):1000–1007

doi: 10.1210/jc.2006-1486

P450c17 Deficiency: Clinical and Molecular Characterization of Six Patients

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Context: The characteristics of P450c17 deficiency include 46,XY disorder of sex development, hypertension, hypokalemia, and lack of pubertal development.

Objective: To better understand this rare enzymatic deficiency, we analyzed the *CYP17A1* gene in six affected patients.

Design and Patients: We examined six patients, five 46,XY, and one 46,XX (age 9–29 yr) with complete lack of masculinization (female infantile external genitalia, no uterus) and delayed puberty, respectively, and different degrees of hypertension.

Main Outcome Measurements: Genotype-phenotype correlation was measured.

Results: Four homozygote mutations were identified by direct sequencing of the CYP17A1 gene corresponding to an alanin 302-proline (A302P) exchange; the loss of lysine 327 (K327del); the deletion of glutamate 331 (E331del); and the replacement of arginine 416 with a histidine (R416H). Both P450c17 activities were abolished in all the mutant proteins, except one, when expressed in COS1 cells. The E331del-mutated P450c17 retained 17α-hydroxylase activity. The mutant proteins were normally expressed, suggesting that the loss of enzymatic activity is not due to defects of synthesis, stability, or localization of P450c17 proteins.

Conclusion: These studies confirm lack of masculinization in 46,XY individuals as the pathognomic sign of the complete P450c17 deficiency. In XX individuals P450c17 deficiency should be considered in cases of delayed puberty. Age of onset and the severity of hypertension do not seem to be constant. Careful examination of long-term follow-ups in two of our patients suggested to us that estrogen treatment in P450c17-deficient patients might worsen the enzymatic defect, leading to aggravation of the hypertension. **(*J Clin Endocrinol Metab* 92: 1000–1007, 2007)**

THE MICROSOMAL ENZYME P450c17 catalyzes two reactions: the 17α-hydroxylation of progesterone and pregnenolone and the subsequent cleavage of the C17–20 carbon bond to produce dehydroepiandrosterone (DHEA) and androstenedione. Whereas only 17α-hydroxylase activity is necessary for the production of corticosteroids, both activities of P450c17 are required to synthesize sex hormones.

17, 20-Lyase activity, unlike 17α-hydroxylase activity, is dependent on phosphorylation of P450c17 (1, 2) and is enhanced by the interaction with redox partners (3, 4).

The enzyme P450c17 is encoded by a single gene, *CYP17A1*, mutations of which may lead to complete combined 17α-hydroxylase/17, 20-lyase deficiency or isolated 17, 20-lyase deficiency. The characteristics of 17α-hydroxylase/17, 20-lyase deficiency include hypertension, hypokalemia, lack of pubertal development, and 46,XY disorder of sex development (DSD). However, in genotype-phenotype correlation studies, a remarkable variation in the severity of the disorder was noted (5). To contribute to the understanding of P450c17 enzymatic deficiencies, we analyzed the *CYP17A1* gene in six patients with such rare defect. Four mutations were identified and their functional consequences were investigated in expression studies.

First Published Online December 27, 2006

Abbreviations: DHEA, Dehydroepiandrosterone; DSD, disorder of sex development; K_M, Michaelis constant; PRA, plasma renin activity; V_{max}, maximal velocity; WT, wild type.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Patients and Methods

Patients

Patients 1 and 2 are siblings born from consanguineous parents. They were raised as girls and presented at 16 and 18 yr of age because of lack of pubertal development. They had prepuberal female external genitalia but no uterus. Their karyotype was 46,XY. Blood pressure was normal.

Patient 3 presented at age 9 yr because of hypertension (160–170/100–110 mm Hg). At physical examination she was a prepuberal girl with normal infantile female external genitalia but with a blind ending vagina. Her karyotype was 46,XY. Absence of uterus and annexes was revealed by ultrasound imaging and confirmed by laparoscopy. This exploration also suggested the presence of undescended testes at the internal inguinal rings. A bilateral gonadectomy performed at the age of 12 yr confirmed the testicular nature of the gonadal tissue.

Patients 4 and 5 are two siblings 29 and 25 yr of age evaluated for primary amenorrhea, hypokalemia, and hypertension (200/120 mm Hg in the older sibling and 180/110 mm Hg in the younger one). The external genitalia were of female appearance with a blind vagina. A pelvic ultrasound showed rudimental annexes with gonadal-like structures.

Patient 6 is a girl who was evaluated at age 17 yr because of delayed puberty (Tanner stage 1). Physical examination revealed the absence of breast development and of pubic or axillary hair. Blood pressure was normal in several occasions and the karyotype was 46,XX. Ultrasonography and magnetic resonance imaging revealed the presence of an

infantile uterus and a large polycystic ovarian mass (Fig. 1, A and B). Treatment with ethynylestradiol (16 μg) was initiated to induce the development of secondary sexual characteristics. The treatment also induced a significant reduction of the ovarian mass (Fig. 1C).

The main clinical features of the patients are summarized in Table 1. Hormonal values, assayed using commercially available kits and confirming the diagnosis of P450c17 enzymatic deficiencies, are summarized in Table 2. ACTH tests corroborated the diagnosis in patients 1, 2, and 3 (data not shown). Urinary steroid profiles were carried out as previously described (6). The diagnosis of P450c17 deficiency is based on the lack of androgen metabolites (androsterone and etiocholanolone) and the elevation of mineralocorticoid metabolites [tetrahydrocorticosterone and its isomer (allotetrahydrocorticosterone); tetrahydrocompound A], and the progesterone metabolites 16-OH-pregnenolone and pregnandiol.

Mutation analysis

After obtaining informed consent, genomic DNA was extracted from peripheral blood leukocytes using a DNA blood and cell culture kit (QIAGEN GmbH, Hilden, Germany) and used to perform PCR exonic amplification of the CYP17A1 gene as previously described (3). The PCR products were sequenced using the Big Dye terminator cycle sequencing kit and analyzed on an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA). Primer sequences are available on request. Once mutations were identified, the corresponding PCR fragments obtained from genomic DNA of 50 normal subjects (100 alleles; 30 Caucasian, eight of whom from Turkey; 10 Asian; 10 blacks, two of whom from Central America; and two Hispanic) were also sequenced to assess ethnic or common polymorphisms.

Expression studies

After addition of an N-terminal myc tag, the *CYP17A1* cDNA was inserted into a pcDNA3.1 vector. Mutant cDNAs were constructed using the QuikChange II site-directed mutagenesis kit from Stratagene (La Jolla, CA). Introduction of the mutations was confirmed by sequencing. Five × 10^4 COS1 cells were initially seeded in DMEM medium, let grow to 80–90% confluence, and then transfected with wild-type or mutant cDNA using TransFast transfection reagent (Promega, Madison, WI). Nine microliters of transfection reagent per microgram DNA per plate). To monitor enzymatic activity of the wild-type (WT) and mutant P450c17, we used a procedure modified from that reported elsewhere (7). Briefly, transfection efficiency was monitored by cotransfection with a β-galactosidase expression plasmid (Promega pSV-β-galactosidase control vector, molar ratio CYP17A1: β-gal = 2:1). β-Gal activity was measured using the β-galactosidase enzyme assay system (Promega). The β-gal milliunits were used to correct the enzyme kinetic data for the transfection efficiency. Forty-eight hours after transfection, steroidogenic precursors (progesterone for 17α-hydroxylase activity and 17OH-pregnenolone for 17, 20-lyase activity) were added at the concentration of 0.1, 0.3, 1, 3, 10, and 30 μM. Six hours after addition of the precursor, supernatants were removed and kept frozen at −20 C until measured. Steroids were extracted in 1:1 heptane to ethylacetate. The secreted steroids, 17OH-progesterone (17α-hydroxylase activity) and DHEA (17, 20-lyase activity), were measured in duplicates by RIA using kits from Diagnostic Product Corp. (Los Angeles, CA). Given the possible problems of RIA use for such assay (8), linearity of the RIA measurements in this system was proven by dilutions (1, 1:2, 1:4, and 1:10; $r^2 = 0.9904$, data not shown) of samples derived from COS1 cells transfected with the WT *CYP17A1* cDNA. All values are expressed as mean ± SD and represent the results of three independent experiments.

Western blot analysis was performed following standard procedures using antimyc antibodies (all constructs, 9E10) and polyclonal rabbit antihuman antibodies kindly provided by Professor Michael R. Waterman (Nashville, TN; E331del, R416H). Total protein determination was carried out using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). One hundred micrograms of total proteins were loaded. The same amount of total protein extracted from NCI-H295R human adrenal carcinoma cells was used as a positive control.

Immunofluorescence

COS-1 cells were grown on glass coverslips and transfected with the wild-type or mutant myc-tagged *CYP17A1* cDNAs. Forty-eight hours after transfection, the cells were fixed for 10 min in methanol at −20 C and blocked for unspecific binding in PBS containing 0.5% powder milk for 30 min at room temperature. The cells were incubated with an antibody against the myc epitope (9E10, dilution 1:100) for 60 min at room temperature followed by a goat antimouse Cy3-conjugated secondary antibody (dilution 1:200; Jackson ImmunoResearch, West Grove, PA) for 60 min. Nuclei were stained in a 1:10,000 dilution of stock solution (Hoechst, Frankfurt, Germany). Coverslips were mounted on glass plates, embedded in Kaiser's glycerol gelatin for microscopy (Merck, Darmstadt, Germany), and examined using an Axioskop microscope (Zeiss, Hilden, Germany).

The study was carried out according to the guidelines of the institutional review board.

Results

In patients 1 and 2, sequencing of the *CYP17A1* gene revealed the presence of a homozygous G to C transversion in exon 5, which leads to the replacement of the alanin at position 302 by a proline (data not shown). To assess the func-

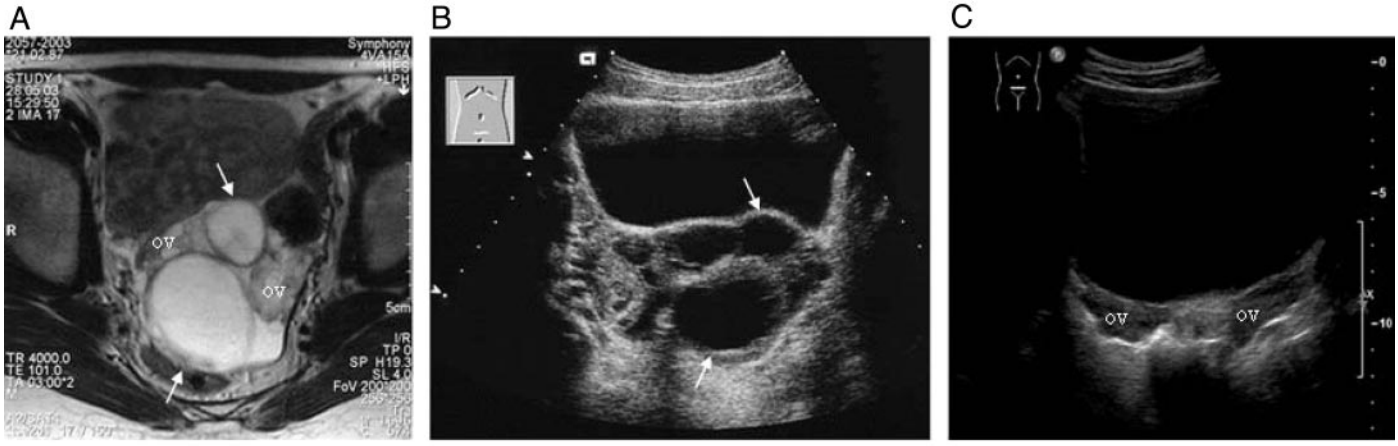


FIG. 1. Abdominal imaging in patient 6. A, Magnetic resonance imaging at presentation (ov = ovary): hypoplastic uterus, normal vagina, ovarian masses indicated by *arrows* (biggest mass, 4 cm diameter). B, Ultrasonography at presentation: uterus 3.5 × 1.4 cm, polycystic mass with a biggest cyst 5 × 3.7 cm with a thick wall (2–3 mm). The ovaries are not well delimited. C, Ultrasonography after 15 months of estrogen therapy (16 μg estradiol), showing reduction of the ovarian masses and signs of uterine maturation: uterus, 5 cm; right ovary, 3.6 × 3.2 × 1.6 cm; left ovary, 5 × 3.6 × 1.5 cm; left mass, 2.8 cm diameter.

TABLE 1. Clinical features of P450c17-deficient patients

Patient	Age at diagnosis (yr)	Karyotype	External genitalia	Sex of rearing	BP (mm Hg)	K (mmol/liter)	Na (mmol/liter)	Special features	P450c17 mutation
1	18	46,XY	F	F	120/70	3.4	141	None	A302P
2	16	46,XY	F	F	120/70	3.1	145	None	A302P
3	9	46,XY	F	F	170/110	2.8	149	None	K327del
4	29	46,XY	F	F	200/120	1.8	150	None	E331del
5	25	46,XY	F	F	180/110	3.2	142	None	E331del
6	17	46,XX	F	F	100/70	3.8	139	Large ovarian mass	R416H

BP, Blood pressure.

tional consequence of the mutation, COS1 cells were transfected with the expression vector pcDNA3 containing mutant or wild-type *CYP17A1* cDNA. The expression of the A302P mutant in COS1 cells showed a complete loss of 17α-hydroxylase as well as 17, 20-lyase activity (Fig. 2).

Patient 3 was found to carry a homozygous 3-bp deletion in exon 6 of *CYP17A1* leading to the loss of a lysine at position 327 [or 325 or 326 (data not shown)]. The mutant protein expressed in COS1 cells is completely inactive (Fig. 2).

Patients 4 and 5 bore a homozygous 3-bp (GAG) deletion in exon 6, leading to the deletion of a glutamate on position 331 (or 330) in the P450c17 protein. The father is heterozygote carrier of the mutation (data not shown). Transfection of the mutant cDNA in COS1 cells led to the synthesis of an enzyme with no significant 17, 20-lyase activity, compared with the WT (Fig. 2B). Although at lower progesterone concentrations (0.1–0.3 μM), 17α-hydroxylase activity reaches merely 1.5–2%, compared with WT, at higher substrate concentrations (1–30 μM), this mutant P450c17 retains significant 17α-hydroxylase activity (Fig. 3A).

Patient 6 carried a homozygous G to A transition in exon 8 with the consequent replacement of arginine 416 by a histidine residue (data not shown). Both activities of P450c17, 17α-hydroxylase and 17, 20-lyase activity, were found to be totally abolished in the mutant protein when expressed in COS1 cells (Fig. 2). This mutation was recently identified in a 46,XX Caucasian patient with P450c17 deficiency (9). The absence of any of the mutations in the *CYP17A1* gene of 50 control subjects from different ethnicities suggests that the rearrangements are not polymorphisms. The mutated residues are conserved among several species.

Although classical enzyme kinetic is not possible in a whole-cell system, putative apparent Michaelis constant (K_M) and maximal velocity (V_{max}) were calculated for all the experimental conditions. The results of such calculations confirmed the impairment of 17, 20-layse activity in all cases, as summarized in Fig. 3, B and D. 17-α-Hydroxylase activity was also impaired in all cases except one (Fig. 3, A and C). Based on these calculations, E331del appears to be at least as efficient as the WT in converting progesterone to 17OH-progesterone (V_{max}/K_M: WT 21.1, E331del 29.5).

Immunofluorescence microscopy demonstrated that the mutant proteins (A302P, K327del, and R416H) were expressed in the cytoplasm of transfected COS1 cells in a fashion that was qualitatively similar to the wild-type protein, suggesting that the loss of enzymatic activity is not due to significant defects of synthesis, stability, or localization of the P450c17 proteins (Fig. 4A). These results were confirmed by a second assay, *i.e.* Western blot analysis using either the antimyc tag or the specific antihuman P450c17 antibody (Fig. 4, B and C).

Discussion

The possibility of studying the *CYP17A1* gene of several patients affected by the rare 17α-hydroxylase/17, 20-lyase deficiency is an effective tool to clarify the molecular mech-

TABLE 2. Plasma basal hormonal values in P450c17-deficient patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Normal values (adult female)
Testosterone (nmol/liter)	n.d.	n.d.	0.07	n.d	0.1	<0.1	0.3–3.8
Androstenedione (nmol/liter)	0.4	0.7	<0.03	0.3	1.3	<0.73	1.4–7.9
DHEA (nmol/liter)	2.4	4.9				2.9	9.8–26.7
DHEA sulphate (μmol/liter)			<0.3	<0.3	0.8	0.9	2.17–15.2
17OH-progesterone (nmol/liter)	7.0	3.9	<0.2	7.5	1.4	12.4	0.3–9.5
Progesterone (nmol/liter)	204	213	1.6	34.5	30.1	152	0.3–1
LH (mIU/ml)	67	29	76	41	43	17.6[a]	5–25
FSH (mIU/ml)	46	22	92	93	82	6.4[a]	4–20
ACTH (pmol/liter)	21.6	41.4	12	33	127		4.4–22.2
Aldosterone (pmol/liter)							
Supine			3.4	62	139	624	28–445
Upright				77	167		111–861
PRA (ng/ml·h)							
Supine			0.13	0.2	1.8	0.2	0.15–2.33
Upright			0.01	0.3	3.8		1–5.7

n.d., Not detectable.

[a] Normal values for pubertal stage (Tanner 1): LH, 1.6 or less; FSH, 2.75 or less.

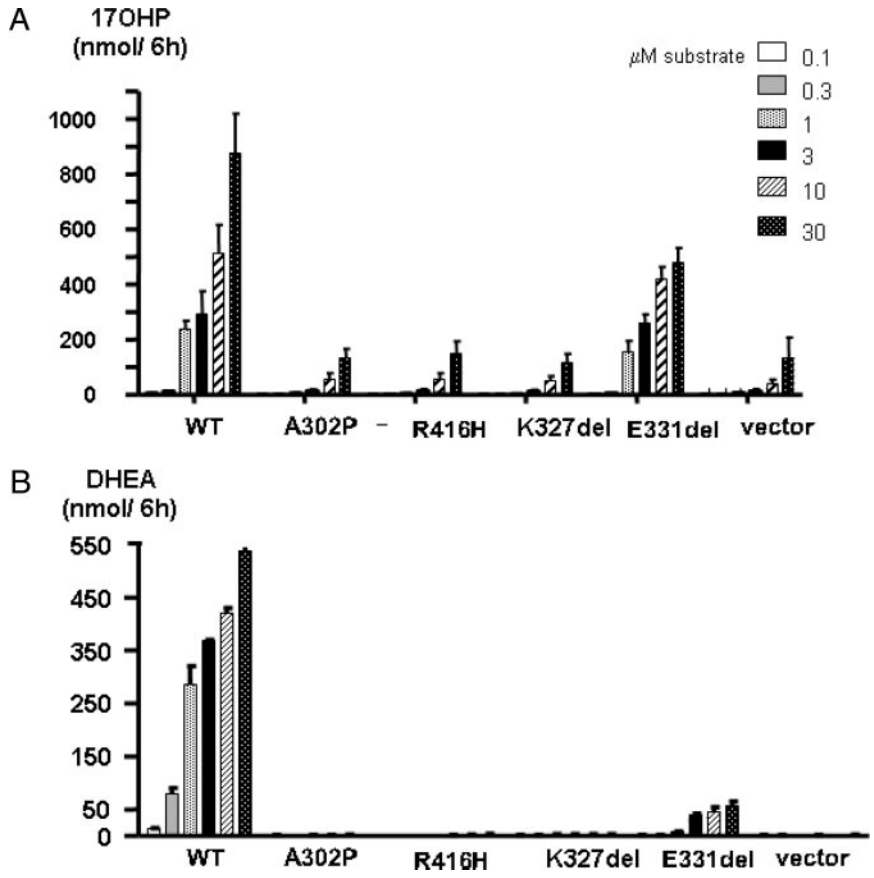
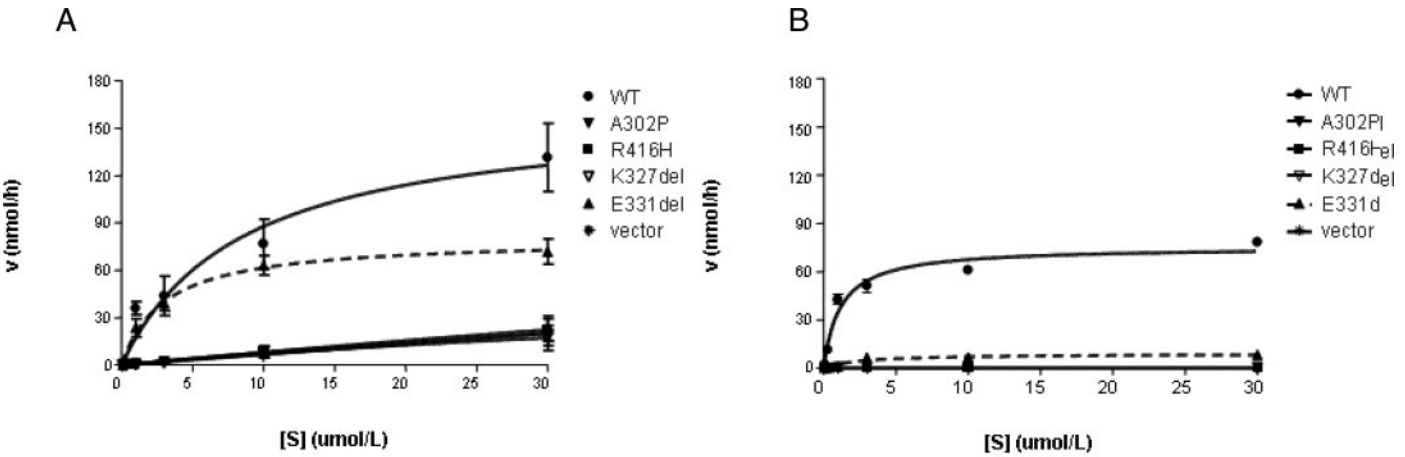


FIG. 2. Production of 17-OH progesterone (17OHP) as a measure of 17α-hydroxylase activity (A) and DHEA (B) as a measure of 17, 20-lyase activity after 6 h incubation with different concentration (0.1, 0.3, 1, 3, 10, and 30 μM) of precursors (progesterone and 17OH-pregnenolone, respectively) in COS1 cells transfected with empty vector (vector), WT, and mutated *CYP17A1* cDNAs. The data represent mean ± SD of three independent experiments.

anisms of the disease and also gather more informations on the structure-function relationship of this protein. P450c17 can be considered as the qualitative regulator of steroidogenesis by determining which kind of steroids will be produced (10): mineralocorticoid in which P450c17 is absent, glucocorticoids when 17α-hydroxylation is active, and sex hormones when 17, 20-cleavage takes place. Thus, knowledge concerning P450c17 will be of great impact for the understanding of hypertension, adrenarche, puberty, and hyperandrogenism, with obvious implications for fertility.

Two main issues are confirmed by our molecular, clinical, and enzymatic studies of mutant P450c17: in 46,XY individuals with the complete deficiency-impaired masculinization is the constant clinical hallmark of this DSD. In 46,XX individuals, in whom the diagnosis seems to be infrequently made (2, 5, 11, 12), it is mandatory to include this rare disease in the differential diagnosis of delayed puberty in girls because a missed diagnosis can lead to severe hypertension and reproductive dysfunction (13), Intriguingly, although P450c17 is the first hypertensive defect of steroidogenesis to be identified (14), age of onset and severity of hypertension do not seem to be as consistent. Two obvious possibilities can be considered to explain the differences in the patients' hypertension. The easiest explanation is dietary: the patients with normal blood pressure may simply be on low-salt diets. Although no main cultural differences in salt intake between the patients are known, we have no evidence that this is the case because a systematic salt-balance study [24 h urine for Na and creatinine, with simultaneous measurement of deoxycorticosterone, aldosterone, and plasma renin activity (PRA) in blood] was not done.

The second possibility is that the *CYP17A1* mutations in patients 1, 2, and 6 are functionally different from those in patients 3, 4, and 5. Patients 1, 2, and 6 have the missense mutations A302P or R416H. By contrast, patients 3, 4, and 5 have amino acid deletions. Whereas these remain in frame, they may have a more deleterious effect on the three-dimensional structure of the enzyme and hence disrupt activity further. This hypothesis is consistent with model of the structure of P450c17 (15), although prediction of the precise enzymatic consequences of a mutation from any structure is not trivial. This theory could not be confirmed by our expression studies because all the mutations, with one exception, caused a complete loss of both 17α-hydroxylase and 17, 20-lyase activities. The deletion of E331 constituted a surprise in that it retained 17α-hydroxylase activity at high substrate concentrations despite the severity of the mutation (a deletion) and the serious clinical phenotype. On the other hand, despite DSD and hypertension, these patients showed the mildest abnormalities at the biochemical level (Table 2), suggesting a possible residual 17α-hydroxylase activity able to clear progesterone more efficiently than the other mutants when progesterone concentrations are high. The severity of hypertension in these patients is not clearly explained by these studies. It has to be noted, however, that the high blood pressure is of late onset (age 29 and 25 yr) suggestive of a milder defect. The influence of dietary salt on blood pressure (salt balance) might help to clarify this point.



C 17α-hydroxylase

	WT	A302P	R416H	K327del	E331del	Vector
Vmax (h^{-1})	120	104	222	68	89	2.147e+008
K_m (μmol)	8.52	109	239	77	3.01	4.812e+008
Vmax/K_m	21.1	0.95	0.92	0.88	29.5	0.44 .

D 17,20-lyase

	WT	A302P	R416H	K327del	E331del	Vector
Vmax (h^{-1})	83	0.36	0.43	0.54	10.6	3.576e+008
K_m (μmol)	1.7	0.07	0.74	0.20	2.6	7.250e+008
Vmax/K_m	48.8	5.1	0.59	2.5	4.0	0.49

FIG. 3. Plot of initial velocity of Michaelis-Menten reaction *vs.* substrate concentration (S). Points are plotted for concentrations of 0.1, 0.3, 1, 3, 10, and 30 μM of progesterone (A) and 17OH-pregnenolone (B). Enzyme kinetic data on the 17α-hydroxylase (C) and 17, 20-lyase (D) activities of WT and mutant *CYP17A1*.

Although our assay system (based on whole cells) does not allow classical enzymology experiments, it appears that our kinetic studies support this clinical observation. This may not be totally surprising because there are two paired E residues at 330–331, so loss of one may have only modest action on charge interactions. E330–331 are in the middle of the J-helix, well downstream from the crucial Thr306 in the middle of the I-helix that is the proton donor required for catalysis. At 337–341 there is a relatively unstructured region between the J and J′ helices; because this is close to 330–331, it seems that it may dampen or ameliorate potential effects of E331del on downstream structures. Thus, the E331del mutant should have the three elements needed for at least some catalysis: Thr 306 in the correct location, a redox-partner binding surface (although this is probably somewhat distorted) and a normal heme-binding region. In this view, E331del results in a partial loss of activity by creating a mild conformational disruption that probably affects the redox-partner binding site more than it affects the substrate binding site.

Careful examination and follow-ups of these patients might offer the chance of identifying factors influencing P450c17 activities, with possible impact in their clinical management. For instance, accurate long-term monitoring in two of our patients suggested to us that estrogen treatment in P450c17-deficient patients might worsen the enzymatic defect, as previously implied (16). In patient 3 (46,XY K327del), whom we followed for 20 yr, and in patient 6 (46,XX R416H), the addition of estrogens to the therapeutic regiment appeared to aggravate the defect, in that blood pressure control was less satisfactory (Fig. 5, C and D) and PRA and aldosterone levels decreased (Fig. 5, A, B, and E) due to increased levels of mineralocorticoid precursors (Fig. 5F). These patterns are suggestive of an aggravation of P450c17 defect. Patient 6, who was normotensive at presentation (see Table 1 for representative blood pressure levels), became symptomatic in that she began to suffer from cephalalgias. A 24-h blood pressure profile under estrogenic therapy showed a hypertensive trend (Fig. 5D), although the lack of a similar profile before estrogens prevented us to directly compare blood pressure values. The symptoms were promptly resolved by treatment with the mineralocorticoid antagonist spironolactone. One straight way to check whether estrogens play a role in this phenomenon would be to lift the putative additional estrogen-dependent burden on P450c17 and reevaluate the patient after suspension of the estrogenic therapy. In patient 6, withdrawal of the estrogenic treatment indeed led to a return to the pretreatment status with amelioration of the PRA and aldosterone levels (Fig. 5E) and the production of mineralocorticoid precursors (Fig. 5F). In patient 3, an autoimmune process involving the adrenals (presence of antiadrenal antibodies, elevation of PRA in the presence of low levels of aldosterone, mild hyponatremia; Fig. 5, A–C) prevented us to assess the adrenal status after stopping estrogens.

Estrogens have been previously shown to block androgen production in the gonads by inhibiting, through the estrogen

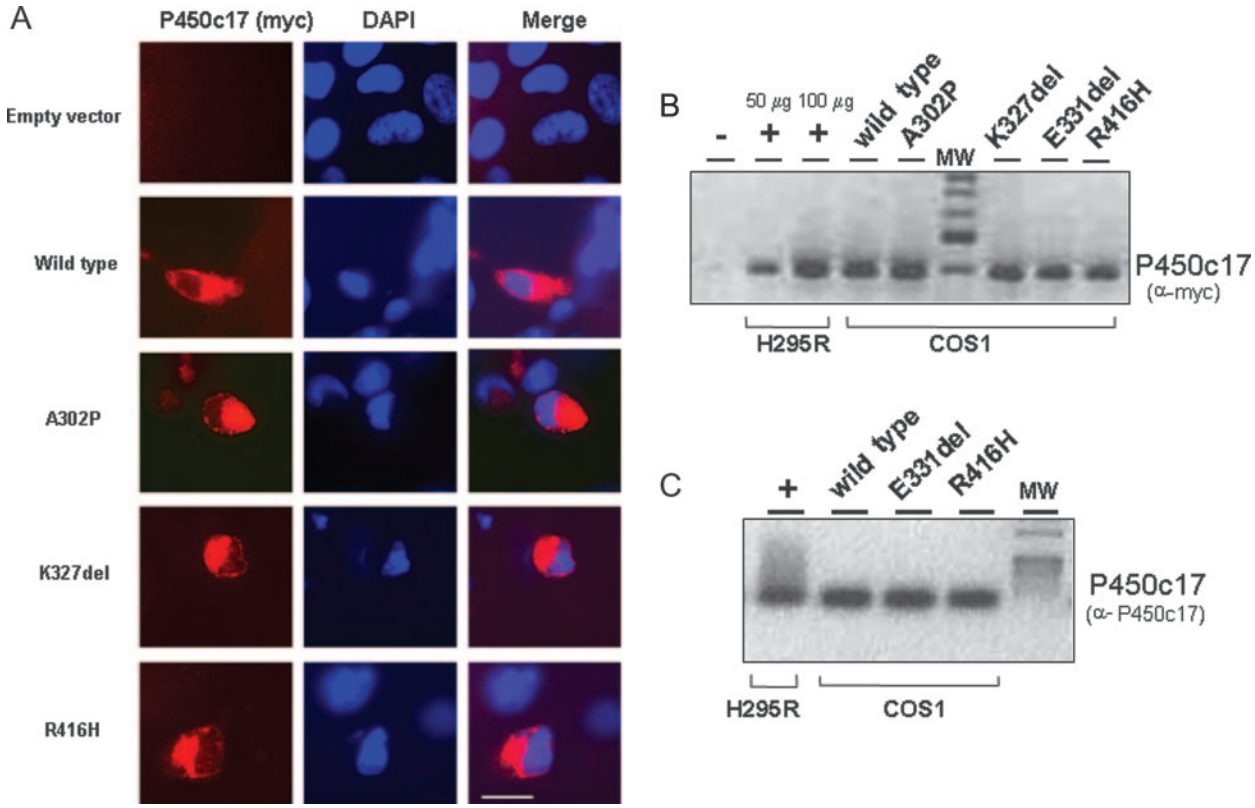


FIG. 4. A, Localization of WT and mutant myc tagged *CYP17A1* in transfected COS1 cells using an antimyc antibody. Cells transfected with an empty vector serve as negative control. The *white line* represents 20 μm scale. B, Western blot analysis using antimyc antibodies or anti-P450c17 (C) of 100 μg total protein from COS1 cells transfected with normal (WT) and mutant *CYP17A1* cDNAs and human H295R adenocarcinoma [as positive control (+)] were blotted. −, No extract. DAPI, 4′,6′-Diamino-2-phenylindole; MW, molecular weight.

receptor, expression and activity of P450c17. Because adrenal cortex also expresses estrogen receptor-α, it is possible that estrogens exert the same effect in this organ. Based on an elegant study in monkeys, Wood *et al.* (17) suggested that (exogenous) estrogens (oral contraceptives and conjugated estrogens) divert adrenal steroidogenesis toward cortisol and away from androgen synthesis, ultimately leading to androgen-deficient hypercortisolemic state. This also seems to be true in humans, as demonstrated by studies in women under oral contraceptives (18–20) or postmenopause estrogen replacement therapy (21–25). If cortisol cannot be synthesized, as in the case of P450c17 deficiency, the putative estrogen-driven detour would be further switched toward the only pathway that is still intact, *i.e.* the mineralocorticoid synthetic route. That would lead to an apparent worsening of the enzymatic defect under estrogens that is exactly what we observed in our patients.

Patient 6 (46,XX R416H) represents one of the fewer genetically female P450c17-deficient patients and might help the clinician to evaluate reproductive consequences of this deficiency in women. Her ovarian phenotype was severe, in that she presented with a gonadal mass, most likely caused by acyclic hyperstimulation due to the elevated levels of gonadotropin (Fig. 1, A and B), promptly reduced by estrogen treatment (Fig. 1C). This phenomenon was already observed in another girl affected by P450c17 deficiency (26), whereas gonadal cysts were never reported in 46,XY patients. Interestingly, the most recently reported 46,XX patient affected by P450c17 deficiency (bearing a compound heterozygosity R125Q/R416H) underwent oophorectomy for ovarian torsion at young age (9), suggesting that the ovarian phenotype must be carefully evaluated in these patients. The ovarian abnormalities and the endometrial dysfunction (due to elevated circulating progesterone levels) might be an additional hurdle in the induction of pregnancy in these patients. That poses a dilemma concerning the therapeutical management of 46,XX P450c17-deficient patients. In fact, these patients need estrogens to induce development of secondary sexual characteristics (as 46,XY patients do) and maintain bone density but also suppress gonadotropin levels, responsible for ovarian hyperstimulation and endometrial dysfunction. In some patients, however, estrogens might aggravate hypertension. As alternative, GnRH agonist in combination with lower doses of estrogens might be used to suppress gonadotropin, induce puberty, and maintain female phenotype. The control of blood pressure can be initially achieved by salt restriction, although mineralocorticoid antagonists might be necessary later in life.

Acknowledgments

The authors are grateful to Professor Walter Miller (University of California, San Francisco) for extremely helpful discussion of the work. We are also indebted to Professor Michael Waterman for the kind gift of the anti-P450c17 antibodies. We acknowledge Bianca Kempken for her excellent technical skills. The authors thank Professor Claus Heizmann for his continuous support.

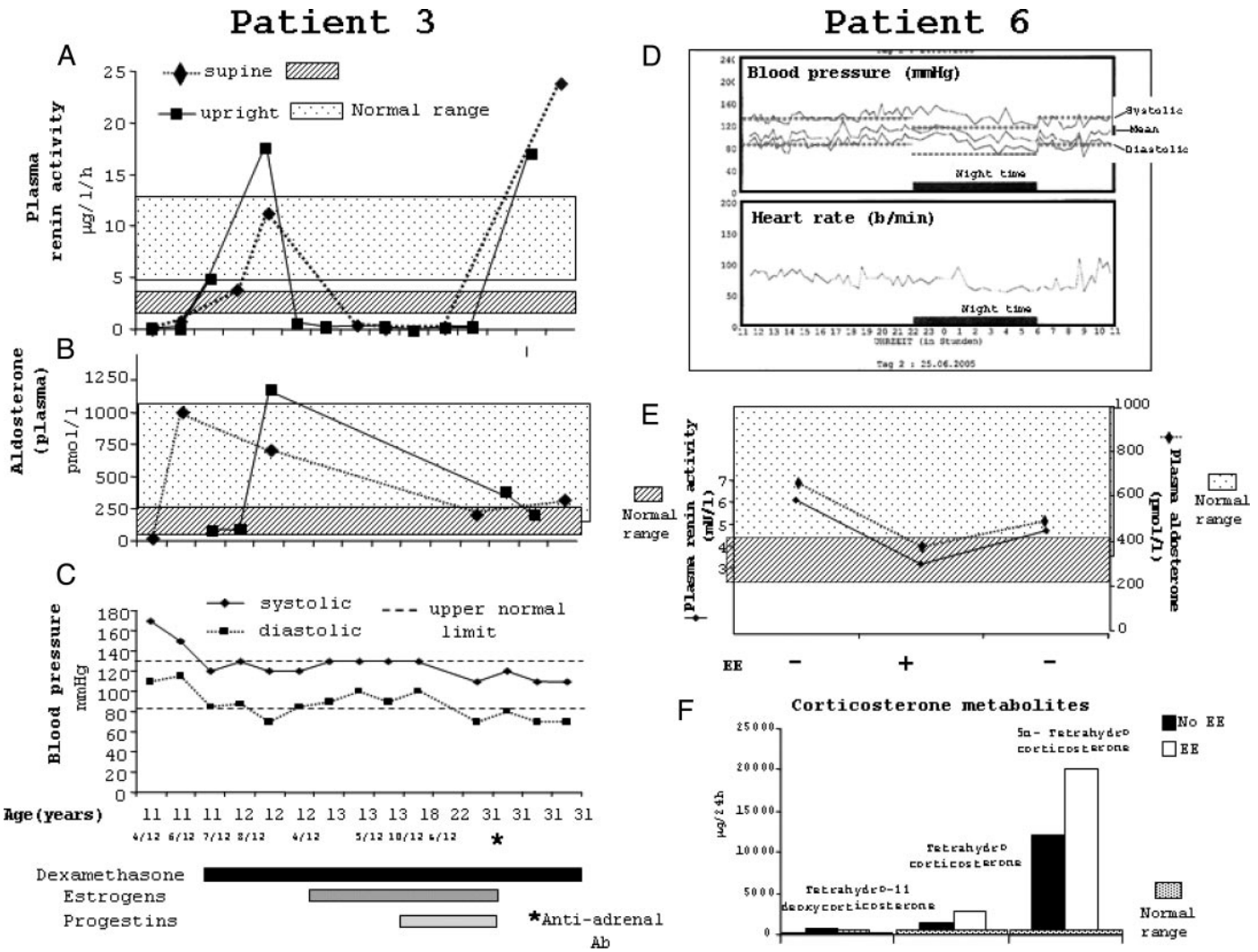


FIG. 5. Estrogen administration might aggravate the enzymatic defect in P450c17-deficient patients: effects on blood pressure and mineralocorticoid system. Twenty-year monitoring of PRA (A), aldosterone levels (B), and long-term blood pressure monitoring (C) in patient 3. Time points are represented as date and age of the patient. Informative time points have been selected. Therapy: dexamethasone 0.5 mg/d; estrogens: ethynyl estradiol 0.1 mg/d; progestin: medroxyprogesterone acetate 5 mg/d. The point of first detection of antiadrenal antibodies is depicted by an *asterisk* (*). D, Overnight blood pressure measurement in patient 6 under estrogen treatment. Effect of oral estrogens (16 µg ethynyl estradiol ee +) on PRA, aldosterone (E), and urinary mineralocorticoid metabolites (F). Withdrawal of the therapy led to a return to the pretreatment status. For patient 6, the follow-up time was 2 yr.

Received July 11, 2006. Accepted December 19, 2006.

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This work was supported by a research grant from the University of Zurich (54181801).

Disclosure Statement: The authors have nothing to disclose.

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