0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(2):898–905

Combined 17α-Hydroxylase/17,20-Lyase Deficiency Caused by Phe93Cys Mutation in the CYP17 Gene

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Seventeen α-hydroxylase/17,20-lyase deficiency is a rare, autosomal recessive form of congenital adrenal hyperplasia not linked to human leukocyte antigen and characterized by the coexistence of hypertension caused by the hyperproduction of mineralocorticoid precursors and sexual abnormalities, such as male pseudohermaphroditism and sexual infantilism in female, due to impaired production of sex hormones. Both 17α-hydroxylase and 17,20-lyase reactions are catalyzed by a single polypeptide, cytochrome P450c17 (CYP17), which is encoded by the *CYP17* gene located on chromosome 10q24-q25. Mutations in the *CYP17* gene have been recognized to cause the 17α-hydroxylase/17,20-lyase deficiency syndrome.

Here, we describe two phenotypically and hormonally affected Italian patients with 17α-hydroxylase/17,20-lyase deficiency. The family history revealed consanguinity of the parents. Linkage and haplotype analyses using microsatellites on chromosome 10q24-q25 demonstrated that the two affected individuals were homozygous at these loci. The mutation screening of the *CYP17* gene identified a new Phe93Cys missense mutation in exon 1. The amino acid substitution is located in a highly conserved region of the protein and is not a polymorphism because it is not present in one hundred normal alleles. *In vitro* functional studies showed that the Phe93Cys mutated CYP17 retains only 10% of both 17αhydroxylase and 17,20-lyase activities, according to the severe phenotype. Our results shed more light on the structure-function relationship of the CYP17 protein indicating that Phe 93 is crucial for both enzymatic activities. (*J Clin Endocrinol Metab* 87: 898–905, 2002)

THE STEROID 17α-HYDROXYLASE/17,20-LYASE is a key enzyme required for the production of cortisol and sex steroids. Both the 17α-hydroxylase and17,20-lyase reactions are known to be catalyzed by a single polypeptide, cytochrome P450c17 (1–3). P450c17 is expressed in several steroidogenic tissues (4, 5), including adrenal cortex, ovary, and testis.

Congenital adrenal hyperplasia resulting from 17α-hydroxylase/17,20-lyase deficiency is a rare autosomal recessive disease, not linked to human leukocyte antigen (6, 7). It is characterized by the presence of hypertension due to an excess of mineralocorticoids other than aldosterone associated with sexual abnormalities such as male pseudohermaphroditism or sexual infantilism in females (8–11). The affected enzyme is encoded by the *CYP17* gene mapped to chromosome 10q24-q25 (12–15).

The present report describes biochemical and molecular studies performed in two related individuals affected by complete 17α-hydroxylase/17,20-lyase deficiency syndrome (17-OHDS). The molecular analysis of the *CYP17* gene allowed us to identify a novel Phe93Cys missense mutation.

Case Reports

Patient 1

A 22-yr-old female patient born from consanguineous parents (Fig. 1) presented with primary amenorrhea, sexual infantilism, and hypertension. On physical examination sexual hair was completely absent, blood pressure was 170/105 mm Hg, and infantile genitalia were present. Abdominal computed tomography scan showed bilateral adrenal hyperplasia, small uterus measuring 29 and 13 mm in the diameters, and enlarged ovaries with multiple cysts, the biggest being 4 cm in the diameter. Her karyotype was 46,XX. Serum sodium, blood urea nitrogen, and creatinine were normal, and her potassium level was 2.87 mmol/liter. Blood pH was 7.452. The measurement of plasma and urinary steroids confirmed the suspicion of combined 17-OHDS (Tables 1 and 2). Therapy with dexamethasone was started, followed by the addition of conjugated estrogens. On dexamethasone therapy, plasma renin activity (PRA) and potassium levels increased to normal and blood pressure and blood pH fell to normal (Table 3).

Patient 2

The family history obtained from patient 1 revealed that patient's sister suffered from primary amenorrhea, sexual infantilism, and hypertension. She was an 18-yr-old patient who had been raised as a girl. When she was 17 yr old, she had been admitted to another hospital because of inguinal pain and presence of lumps in inguinal regions bilaterally. Both inguinal masses were operated, and pathological examination revealed the presence of testes. Based on these findings, the absence of pubic and axillary hair and the 46,XY karyotype, the diagnosis of androgen resistance syndrome was made. The patient was admitted to our hospital 1 yr

Abbreviations: 17-OHDS, 17α-Hydroxylase/17,20-lyase deficiency syndrome; DHEA, dehydroepiandrosterone; PRA, plasma renin activity.

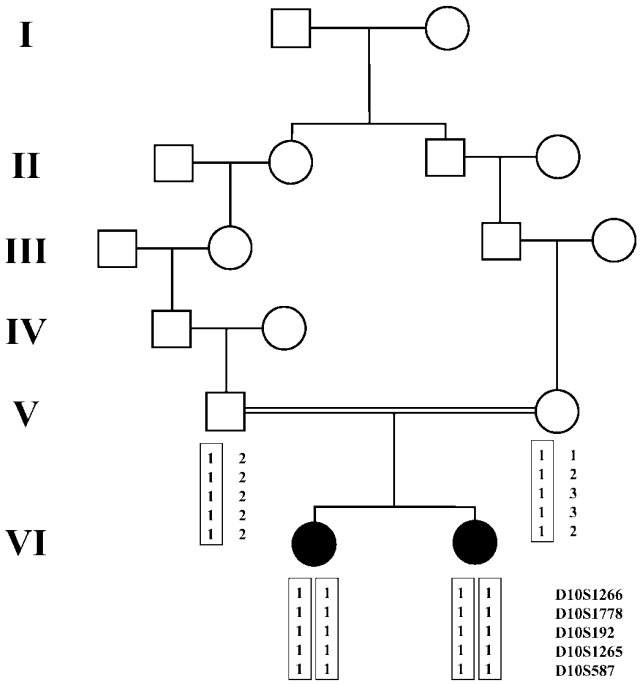

FIG. 1. Family pedigree and haplotype reconstruction for informative markers close to the CYP17 gene. The at-risk haplotype is *boxed*.

later. Physical examination showed female infantile external genitalia and a complete absence of sexual hair and breast development. Blood pressure was 155/110 mm Hg. At abdominal computed tomography scan there was a bilateral adrenal hyperplasia, whereas müllerian structures were absent. Serum sodium, blood urea nitrogen, and creatinine were normal. The potassium level was 3.66 mmol/liter. Blood pH was 7.424. As in the case of patient 1, the measurement of plasma and urinary steroids confirmed the suspicion of combined 17-OHDS (Tables 1 and 2). Therapy with dexamethasone was started, followed by the addition of conjugated estrogens. During therapy with dexamethasone serum levels of blood urea nitrogen, creatinine, and potassium increased. Blood pressure and gas analysis and PRA normalized (Table 3).

Family studies

After informed consent was obtained, we also studied the family of the two affected sisters. The parents, ages 49 and 45 yr, were consanguineous (see Fig. 1). Neither had hypertension or metabolic alkalosis. Both had normal levels of serum potassium. Their serum steroid hormone concentrations are shown in the Table 1.

Materials and Methods

Hormone assays

All serum and urine samples were stored at −30 C until analysis. Serum LH, FSH, and steroid levels and plasma ACTH and renin activity were measured by commercial kits based on RIAs, immunoradiometric assays, electrochemiluminescence immunoassays, and fluoroimmunometric methods. Urinary steroids were assayed by combined gas chromatography/mass spectrometry following the method described by Shackleton (16). Appropriate reference steroids were obtained from Sigma-Aldrich Corp. (Milan, Italy). Assays were performed in basal conditions, after standard ACTH and human CG stimulation tests, during a long-term dexamethasone and dexamethasone plus conjugated estrogen therapy, and 2 wk and 4 months after the cessation of glucocorticoid therapy (Table 3).

Southern blot analysis

Genomic DNA of the four members of family and control DNA were prepared from peripheral blood using the standard method and digested to completion with *Hin*dIII restriction enzyme (Roche Molecular Biochemicals GmbH, Mannheim, Germany). DNA samples were then subjected to electrophoresis on 0.8% agarose gels and blotted onto Hybond-N nylon membrane (Amersham Pharmacia Biotech, Uppsala, Sweden) by the method of Southern blot. The membrane was hybridized overnight with a probe containing the *CYP17* gene that had been labeled with ^{32}P by the random hexanucleotide-primed method. The membranes were washed at 65 C in 1× SSC (0.15 M sodium chloride and 0.015 M sodium citrate) and 0.1% SDS, then exposed to x-ray film with intensifying screens at −70 C for 1–7 d.

Linkage analysis

The polymorphic markers D10S1266, D10S1778, D10S192, D10S1265, and D10S587 were amplified for linkage analysis at 10q24-q25 (17). Fluorescently labeled PCR amplifications were performed in 25-μl reaction volumes containing 100 ng genomic DNA, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 0.01% Tween 20, 0.01% gelatin, 0.01% NP40, 15 pM of both fluorescently labeled and nonlabeled primers, and 1 U *Taq* DNA polymerase (Roche Molecular Biochemicals GmbH). Initial denaturation was for 3 min at 94 C, followed by amplification for 30 cycles with denaturation at 94 C for 30 sec, annealing for 30 sec at the required temperatures, and extension at 72 C for 30 sec. Amplification products were analyzed by GENESCAN software in ABI PRISM 377 DNA sequencer (Perkin-Elmer Corp., Foster City, CA).

PCR and DNA sequencing

Oligonucleotides were designed spanning all eight exons and intron/exon boundaries of the *CYP17* gene. PCR amplification of exons was carried out in a 25-μl reaction volumes containing 100 ng genomic DNA, 15 pM of each primer, 200 μM of each dNTP, 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 1.5 mM $MgCl_2$, and 0.8 U *Taq* DNA polymerase (Roche Molecular Biochemicals GmbH). Initial denaturation was for 3 min at 94 C, followed by amplification for 30 cycles with denaturation at 94 C for 30 sec, annealing for 30 sec at the required temperatures, and extension at 72 C for 30 sec. PCR samples were purified by use of GFX PCR DNA and Gel Band Purification kit (Amersham Pharmacia Biotech) and sequenced in both directions using the Thermo Sequenase dye terminator sequencing pre-mix kit (Amersham Pharmacia Biotech, Cleveland, OH). Data were analyzed using ABI PRISM 377 DNA sequencer (Perkin-Elmer Corp.).

In vitro expression

To study the functional implications of the mutations found, we used a RT-PCR method using CYP17 mRNA ectopically expressed in peripheral blood leukocytes of the patients as described previously (18) The mutated cDNAs were subcloned into a pCMV4 vector and transiently transfected into confluent COS-1 cells using 50 μg Lipofectamine and 10 μg DNA on a 10-cm plate (Life Technologies, Inc., Grand Island, NY). The correctness of the sequence was proven by sequencing. The transfection efficiency ranged from 40–60%. Forty-eight hours after transfection, steroidogenic precursors (pregnenolone and progesterone for 17α-hydroxylase activity and 17α-hydroxypregnenolone for 17,20-lyase activity) were added at a concentration of 1 μmol/liter after suspension in 1× phosphate buffer. Six hours after its addition, supernatant was removed and kept frozen at −20 C until measured. To standardize the steroid production, cells were lysed in 1× PBS, 1.5 mmol/liter $MgCl_2$, 1 mmol/liter ethylenediamine tetraacetate, 1% Triton-X, and 10% glycerol in the presence of protease inhibitors (34 μg/ml phenylmethane-

TABLE 1. Serum steroid hormone concentrations in the patients with 17α-hydroxylase/17,20-lyase deficiency

	Patient 1	Patient 2	Father	Mother	Normal adult values	
					Males	Females
BUN (mmol/liter)	7.9	11.1	19.3	21.1	3.6–17.9	
Creatinine (μmol/liter)	69.0	86.6	158.2	76.9	44.2–114.9	
Na^+ (mmol/liter)	143	141	148	144	135–153	
K^+ (mmol/liter)	2.87	3.66	4.44	4.10	3.50–5.30	
pH	7.45	7.42	7.38	7.37	7.35–7.45	
P (nmol/liter)	38.2	34.0	1.5	1.3	0.3–3.8	0.5–4.5
17OH-P (nmol/liter)	3.76	4.76	4.24	2.33	1.27–10.61	0.61–7.88
DHEA (nmol/liter)	2.15	2.98	9.02	11.17	4.86–43.38	2.78–36.44
DHEAS (nmol/liter)	0.03	3.85	1274	1893	5065–12834	3409–9433
Δ_4 (nmol/liter)	0.03	0.03	1.47	1.43	1.99–9.25	1.64–9.35
T (nmol/liter)	0.03	0.03	3.88	0.49	8.32–34.67	0.69–3.12
E2 (pmol/liter)	0.04	12	57	93	ND–162	37–184
Cortisol at 0800 h (nmol/liter)	168	218	66	179	193–690	
Cortisol at 1800 h (nmol/liter)	157	281	NA	NA	55–248	
Aldo supine (pmol/liter)	447	583	NA	NA	21–416	
Aldo upright (pmol/liter)	666	804	641	546	97–832	
PRA supine (ng/liter·sec)	0.003	0.03	NA	NA	0.06–0.78	
PRA upright (ng/liter·sec)	0.003	0.03	0.45	0.09	0.42–1.58	
ACTH at 0800 h (pmol/liter)	23.6	17.0	2.6	2.4	2.2–13.2	
ACTH at 1800 h (pmol/liter)	22.0	28.2	NA	NA	1.3–6.6	
UFC (nmol/24 h)	19	45	39	135	22–166	
LH (IU/liter)	13.60	19.82	16.69	12.81	1.7–8.9	2.4–12.6
FSH (IU/liter)	8.23	40.11	54.84	27.76	1.5–12.4	3.5–12.5

BUN, Blood urea nitrogen; P, progesterone; 17OH-P, 17α-hydroxyprogesterone; DHEAS, DHEA sulfate; Δ_4, Δ_4-androstenedione; Aldo, aldosterone; UFC, urinary free cortisol; ND, not detectable; NA, not assayed.

sulfonylfluoride, 0.7 μg/ml pepstatin, and 5 μg/ml leupeptin; Roche Molecular Biochemicals, Rotkreuz, Switzerland), and protein content was measured using protein assay reagents obtained from Bio-Rad Laboratories, Inc. (Hercules, CA). The secreted steroids [*i.e.* 17α-hydroxyprogesterone (17α-hydroxylase activity) and dehydroepiandrosterone (DHEA) (17,20-lyase activity)] were measured in duplicate by RIA using Diagnostic Products kits (Los Angeles, CA). All values are expressed as the mean ± SD and represent the results of three independent experiments. Western blot analysis was performed using standard procedure (19).

Results

Steroid hormones at first admission

Plasma steroids. Both patients had low normal cortisol basal values and inadequate response of cortisol to ACTH. Serum progesterone was high, and 17α-hydroxyprogesterone was normal. The response of progesterone and that of 17α-hydroxyprogesterone to ACTH were negligible to absent. Serum levels of all the C_{19} steroids were low to undetectable before and after ACTH and human CG stimulation (Tables 1 and 4). In both parents the basal levels of progesterone, 17α-hydroxyprogesterone, and DHEA were normal, whereas serum levels of androstenedione and T were moderately low, predicting their heterozygosity.

Urinary steroids. The basal urinary steroid levels are reported in Table 2. In both patients levels of the urinary metabolites of corticosterone were largely above the normal range. Levels of the urinary metabolites of androstenedione, T, E2, and DHEA were low to undetectable according to their serum concentrations. Levels of the urinary metabolites of pregnenolone and progesterone were increased, whereas urinary metabolites of 17α-hydroxypregnenolone, 17α-hydroxyprogesterone, and cortisol were low. By contrast, both parents had near-normal levels of the urinary metabolites of pregnenolone, progesterone, corticosterone, and cortisol and relatively low levels of the urinary metabolites of androstenedione, T, and DHEA.

Clinical course and steroid hormones after treatment with dexamethasone

Both patients were initially treated with 0.25 mg dexamethasone. Treatment failed to normalize ACTH and aldosterone levels and PRA. Moreover, blood pressure was constantly found above normal values. To achieve a more physiological condition, the dose of the drug was doubled. After several months of this treatment, in which a normalization of potassium, aldosterone, renin activity, and blood pressure was obtained (Table 3), both patients presented claiming the appearance of *striae rubrae* predominantly localized on the abdomen, thighs, and in axillae. Thus, after a 4-month off-treatment period, the daily dose of dexamethasone was reduced and conjugated estrogens (Premarin) were added at a 0.3-mg daily dose for 6 months and 0.625 mg afterward (Table 3). Following this regimen, patient 1 experienced regular menstrual bleeding and breast development, whereas patient 2 experienced breast development also. Hormonal measurements were performed 2 wk and 4 months after the cessation of glucocorticoid therapy (early and late off-treatment, respectively). As shown in Table 3, progesterone and PRA, which had been normalized by treatment, rapidly returned to the values seen in the untreated period, indicating that both adrenal steroids and renin-angiotensin system are ACTH dependent. Moreover, potassium returned to the lower limits of the normal range, sodium remained essentially unchanged, and hypertension recurred.

TABLE 2. Urinary steroid metabolite concentrations

Urinary metabolite	Parent compound	Patient 1	Patient 2	Father	Mother	Normal values
5β-Pregnan-3α-ol-20-one	Preg, P	210	200	5	4	ND
5β-Pregnan-3α,20α-diol	P	2300	1067	24	98	100–2500
Δ_5-Pregnen-3β,20α-diol	Preg	1900	2790	33	26	ND
Δ_5-Pregnen-3β,17α,20α-triol	17OH-Preg	24	78	10	14	40–430
Δ_5-Pregnen-3β,16α,20α-triol	16OH-Preg	900	836	25	27	ND
5β-Pregnan-3α,17α-diol-20-one	17OH-P	44	15	71	42	20–600
5β-Pregnan-3α,17α,20α-triol	17OH-P	68	49	255	125	100–1700
Δ_5-Androsten-3β-ol-17-one	DHEA	0	38	7	70	50–900
Δ_5-Androsten-3β,17β-diol	DHEA	0	8	4	7	70–450
Δ_5-Androsten-3β,16α-diol-17-one	16OH-DHEA	0	27	170	110	65–500
Δ_5-Androsten-3β,16α,17β-triol	16OH-DHEA	0	29	160	17	90–400
5α-Androstan-3α-ol-17-one	Δ_4; T	27	17	356	317	900–3500
5β-Androstan-3α-ol-17-one	Δ_4; T	28	5	95	220	700–3000
5α-Androstan-3β-ol-17-one	Δ_4; T	0	0	2	11	10–200
5α-Androstan-3α,17β-diol	Δ_4; T	0	0	8	2	15–230
5β-Androstan-3α,17β-diol	Δ_4; T	0	0	7	5	15–230
5α-Androstan-3α-ol-11,17-dione	11OH-Δ_4	0	0	190	94	148–650
5α-Androstan-3α,11β-diol-17-one	11OH-Δ_4	13	19	540	177	318–1600
5β-Androstan-3α,11β-diol-17-one	11OH-Δ_4	6	2	104	120	100–550
1,3,5(10)-Estratrien-3-ol-17-one	E2	0	0	0	0	ND
1,3,5(10)-Estratrien-3,17β-diol	E2	0	0	0	0	ND
1,3,5(10)-Estratrien-3,16α,17β-triol	16OH-E2	0	0	0	0	ND
5α-Pregnan-3α,21-diol-11,20-dione	A	15000	3600	158	36	ND
5β-Pregnan-3α,21-diol-11,20-dione	A	2600	17000	180	146	50–250
5β-Pregnan-3α,20α,21-triol-11-one	A	872	1400	25	19	ND
5α-Pregnan-3α,20α,21-triol-11-one	A	660	350	2	9	ND
5α-Pregnan-3α,11β,21-triol-20-one	B	77000	172000	580	320	80–500
5β-Pregnan-3α,11β,21-triol-20-one	B	1400	610	130	140	70–300
5α-Pregnan-3α,11β,20α,21-tetrol	B	672	1900	12	6	ND
5β-Pregnan-3α,11β,20α,21-tetrol	B	150	206	1	2	ND
5β-Pregnan-3α,17α,21-triol-20-one	S	5	10	23	11	10–100
5β-Pregnan-3α,17α,20α-triol-11-one	21-DO-F	1	7	22	5	10–100
5β-Pregnan-3α,11β,17α,20α-tetrol	21-DO-F	34	31	20	3	20–140
5α-Pregnan-3α,17α,21-triol-11,20-dione	E	0	52	84	30	ND
5β-Pregnan-3α,17α,21-triol-11,20-dione	E	225	172	2600	1300	750–2000
5β-Pregnan-3α,17α,20α,21-tetrol-11-one	E	40	12	580	305	180–850
5β-Pregnan-3α,17α,20β,21-tetrol-11-one	E	24	18	280	90	100–620
5β-Pregnan-3α,11β,17α,21-tetrol-20-one	F	17	60	900	624	650–1700
5α-Pregnan-3α,11β,17α,21-tetrol-20-one	F	257	631	1700	390	30–1500
5β-Pregnan-3α,11β,17α,20α,21-pentol	F	21	26	29	50	10–500
5β-Pregnan-3α,11β,17α,20β,21-pentol	F	45	14	208	102	50–300
Δ_4-Pregnen-11β,17α,20α,21-tetrol-3-one	F	0	0	22	10	ND
Δ_4-Pregnen-11β,17α,20β,21-tetrol-3-one	F	0	0	12	12	ND
Δ_4-Pregnen-11β,17α,21-triol-3,20-dione	F	0	0	64	36	17–70

Results are expressed as micrograms per 24 h.

Preg, Pregnenolone; 17OH-Preg, 17α-hydroxypregnenolone; 16OH-Preg, 16α-hydroxypregnenolone; P, progesterone; 17OH-P, 17α-hydroxyprogesterone; 16OH-DHEA, 16-hydroxy-DHEA; Δ_4, Δ_4-androstenedione; 11OH-Δ_4, 11-hydroxy-Δ_4-androstenedione; 16OH-E2, 16-hydroxy-E2; A, 11-dehydrocorticosterone; B, corticosterone; S, 11-deoxycortisol; 21-DO-F, 21-deoxycortisol; E, cortisone; F, cortisol; ND, not detectable.

Molecular analyses

Microsatellites D10S1266, D10S1778, D10S192, D10S1265, and D10S587 located on chromosome 10q24-q25 (17) close to the *CYP17* gene were used expecting to find a region of homozygosity because of the consanguinity in the family (Fig. 1). In fact, the two affected individuals shared the same allele at all these loci, suggesting that both alleles were affected by the same germ-line mutation. The maximum LOD score for informative markers was 1.29.

The *CYP17* gene was screened for mutations in one proband (VI-2) and her father by direct sequencing of the coding region, including the eight exons amplified together with the flanking donor and acceptor splice sites, and the promoter region. Six different nucleotide substitutions were found in both samples, when each sequence was compared with that of the gene in GenBank (accession no. M63871). They included three silent changes (T138C, T195G, and T849C) and two missense mutations, C66G and T278G, responsible for Cys22Trp and Phe93Cys amino acid substitutions in the coding region of exon 1, respectively. Cys22Trp is likely to be a polymorphism, as found in GenBank (accession no. M14564). Therefore, we believed that Phe93Cys was the mutation responsible for the syndrome of 17α-hydroxylase/17,20-lyase deficiency in this family. Several aspects of the present work confirmed our hypothesis. First, segregation analysis in the family demonstrated that both parents were heterozygous whereas the two affected daughters were homozygous, as expected on the basis of linkage analysis data, for the T278G substitution (Fig. 2). Second, the importance of Phe93 was supported by the observation that this amino acid is conserved in all the P450c17 enzymes characterized

TABLE 3. Serum steroid hormone concentrations before and after dexamethasone (Dex) and conjugated estrogen (CE) therapy

Months of Rx		Patient 1										Patient 2									
		0	1	2	4	5	Off early	Off late	3	6	12	0	1	2	4	5	Off early	Off late	3	6	12
Treatment (ng/d)	Dex	0	0.5	0.5	0.5	0.5	0	0	0.25	0.25	0.25	0	0.5	0.5	0.5	0.5	0	0	0.25	0.25	0.25
	CE	0	0	0	0	0	0	0	0.3	0.3	0.625	0	0	0	0	0	0	0	0.3	0.3	0.625
Variable																					
P (nmol/liter)		38.2	26.7	37.5	23.3	18.1	28.6	36.3	25.1	15.3	18.1	34.0	5.3	3.8	3.4	3.1	22.7	23.0	15.3	11.8	8.0
17OH-P (nmol/liter)		3.76	1.61	1.73	1.12	1.45	1.97	3.58	1.70	1.09	1.36	4.76	0.97	0.45	0.61	0.39	1.82	4.76	1.12	0.97	1.09
DHEA (nmol/liter)		2.15	3.09	0.03	3.96	3.19	2.12	1.53	3.30	2.71	2.46	2.98	4.16	3.47	4.09	3.30	2.74	2.98	5.45	2.88	3.37
DHEAS (nmol/liter)		0.03	6.71	2.73	0.03	0.03	0.03	0.03	25.87	12.22	4.21	3.85	4.65	0.03	0.03	3.17	0.03	4.08	23.14	11.75	3.07
Δ_4 (nmol/liter)		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
T (nmol/liter)		0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
E_2 (pmol/liter)		0.04	10	45	60	24	29	8	69	168	178	12	14	12	14	0.04	0.04	0.04	55	34	66
Cortisol at 0800 h (nmol/liter)		168	NA	NA	NA	NA	44	NA	NA	NA	NA	218	NA	NA	NA	NA	196	NA	NA	NA	NA
Aldo upright (pmol/liter)		666	63	129	67	135	148	316	210	29	1101	804	560	160	113	162	268	255	185	162	125
PRA upright (ng/liter·sec)		0.003	0.08	0.40	0.59	2.20	0.01	0.03	1.37	0.46	1.44	0.03	1.92	0.53	0.34	0.73	0.02	0.04	0.05	0.06	0.79
ACTH at 0800 h (pmol/liter)		23.6	1.4	1.6	1.4	1.0	91.2	328.1	3.9	2.8	202.6	17.0	7.9	2.2	1.0	0.6	71.3	109.7	16.4	68.5	8.6
LH (IU/liter)		13.6	14.8	NA	12.1	13	14	18	17.25	15.8	16	19.82	23.4	NA	21.9	25.3	21.4	24	24	20.9	22.9
FSH (IU/liter)		8.23	9.16	NA	7.5	10.4	10.3	15.3	10.51	10.4	9	40.11	44.6	NA	47	55	50	52	52	40.9	38.2

See Table 1 for abbreviations.

to date, including horse, sheep, bovine, guinea pig, rat, mouse, rainbow trout, dogfish, chicken, and frog (Fig. 3). Third, T278G was absent in 50 unaffected, unrelated control individuals.

The *in vitro* expression studies demonstrated that the Phe93Cys mutation did not affect protein translation and stability (data not shown). Transfection of the mutant cDNA in COS-1 cells leads to the synthesis of an enzyme retaining only about 10% activity compared with the wild type, with no significant difference between 17α-hydroxylase and 17,20-lyase activities (Table 5).

Discussion

Since the original description by Biglieri *et al.* (20), over 120 cases of 17α-hydroxylase deficiency have been reported (reviewed in Refs. 8–11). Furthermore, a few cases of 17,20-lyase deficiency have been described in which 17α-hydroxylase activity was normal (8–11). Mutations in the *CYP17* gene have been identified in 28 patients with 17α-hydroxylase/17,20-lyase deficiency. The mutations are of different natures, including deletions, insertions, and single base changes, and are spread throughout the gene. Recently, G to A and G to T substitutions have been described in Japanese patients at position +5 in the splicing donor site in introns 2 and 7, respectively (21, 22). Both patients were suffering from a combined 17α-hydroxylase/17,20-lyase defect.

Here, we report the cases of two sisters suffering from a well documented 17-OHDS. The long-term study of patients and obligate heterozygotes (parents) suggests the following considerations. The hormonal profile observed in basal conditions, characterized by the marked ACTH-driven elevation of all compounds above the block, including the mineralocorticoids produced by the zona fasciculata in the 17-deoxy pathway, the significant reduction of cortisol, and the near complete absence of Δ_4- and Δ_5-androgens (Tables 1 and 2), strongly indicates a severe form of combined 17α-hydroxylase/17,20-lyase deficiency. Serum and urine determinations performed in the parents also showed some abnormalities (slight reduction of Δ_4- and Δ_5-compounds below the block, increased ratio of C-21,17-deoxy to C-21,17-hydroxy urinary metabolites, and suppressed PRA in the mother) (Tables 1 and 2). Moreover, the administration of exogenous ACTH, which did not evoke any significant rise of 17-hydroxylated steroids in the patients, elicited a normal production of cortisol and a near-normal increase of Δ_4- and Δ_5-androgen precursors in both parents. Thus, biochemical data obtained in this family appear to meet the general criteria proposed for the identification of heterozygous siblings (23).

Most patients with 17α-hydroxylase deficiency have absent or subnormal production of aldosterone. It has been suggested that the inhibition of aldosterone biosynthesis is mediated by the increased levels of mineralocorticoids, which lead to suppression of renin-angiotensin system via an increased reabsorption of sodium and increased blood volume (24). By contrast, in our patients aldosterone levels were high in the supine position despite suppressed renin and

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