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A novel compound heterozygous mutation of K494_V495 deletion plus R496L and D487_F489 deletion in extreme C-terminus of cytochrome P450c17 causes 17α-hydroxylase deficiency

Long-Shyong Lee [a,b,*], Wei-Jane Shu [a], Chen-Ming Wu [a], Chia-Hsing Hsieh [b], Su-Mei Chen [c], Chaur-Jong Hu [d,e], Wei-Yi Chen [f], Bon-chu Chung [f]

[a] *Department of Clinical Pathology, Jen-Ai Branch, Taipei City Hospital, Taipei, Taiwan*
[b] *Department of Endocrinology/Metabolism, Jen-Ai Branch, Taipei City Hospital, Taipei, Taiwan*
[c] *Department of Nuclear Medicine, Jen-Ai Branch, Taipei City Hospital, Taipei, Taiwan*
[d] *Departments of Neurology, Jen-Ai Branch, Taipei City Hospital, Taipei, Taiwan*
[e] *Departments of Molecular Medicine, Jen-Ai Branch, Taipei City Hospital, Taipei, Taiwan*
[f] *Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan*

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Abstract

17α-Hydroxylase deficiency is a rare disease caused by mutation of the *CYP17* gene, resulting in hypertension, hypokalemia, female sexual infantilism or male pseudohermaphroditism, low blood cortisol and low plasma renin activity. Herein, we report a female Taiwanese with 17α-hydroxylase deficiency. The *CYP17* genes of this patient and five members of her family were analyzed by PCR-direct sequencing. One allele of the patient contains a 9-bp (c. 1459–1467 GACTCTTTC: D487, S488, F489) deletion, which is prevalent in Southeast Asia. The other allele has a 6-bp (c. 1480–1485 AAGGTG: K494, V495) deletion and an R496L (c. 1487 G>T) missense mutation, which is a novel mutation. Site-directed mutagenesis, in vitro expression and functional analysis in HEK-293T cells showed that this novel mutation [K494_V495 Del; R496L] resulted in complete loss of 17α-hydroxylase and 17,20-lyase activity. Thus this novel mutation in the extreme C-terminus abolishes enzyme activity, and when accompanied by a 9-bp deletion at codons 487–489 in the other allele, results in 17α-hydroxylase/17,20-lyase deficiency in this patient.

Keywords: Congenital adrenal hyperplasia; 17α-Hydroxylase deficiency; *CYP17*; Mutation

1. Introduction

17α-Hydroxylase deficiency is a relatively rare disease, clinically presented as hypertension, hypokalemia, male pseudohermaphroditism or female sexual infantilism, low plasma cortisol with elevated ACTH, and suppressed renin-angiotensin system and aldosterone production (Biglieri et al., 1966). In congenital adrenal hyperplasia, the reported prevalence of 17α-hydroxylase deficiency was the third after those of 21α-hydroxylase and 11β-hydroxylase deficiencies in studies from the Middle East, United States and Western Europe. In Japan and Korea, congenital lipoid adrenal hyperplasia was the second and 17α-hydroxylase deficiency was the third in prevalence (Miller, 2004). This disease is caused by the defect of a single cytochrome P450c17 (*CYP17*) enzyme. This enzyme sequentially catalyzes two reactions: the 17α-hydroxylation of steroid C21 (pregnenolone to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone), and followed by the 17,20-lyase activity which cleaves the C17,20 carbon bond to form C19 steroids (mainly 17α-hydroxypregnenolone to dehydroepiandrosterone and minimally 17α-hydroxyprogesterone to androstenedione) in adrenal glands and gonads (Auchus et al., 1998; Lee-Robichaud et al., 1995). Therefore, P450c17 is essential for the production of glucocorticoids and sex hormones. In adrenal glands, two other tissue specific enzymes, P450c21 and P450c11, catalyze the conversion of progesterone to produce mineralocorticoids and the conversion of

* Corresponding author at: Department of Clinical Pathology, Jen-Ai Branch, Taipei City Hospital, No. 10, Sec. 4, Jen-Ai Road, Taipei city 106, Taiwan. Tel.: +886 2 27093600x5102/5139; fax: +886 2 27035249.
E-mail address: lls045@yahoo.com.tw (L.-S. Lee).

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17α-hydroxyprogesterone to produce glucocorticoids. P450c17 is encoded by *CYP17* (Chung et al., 1987; Picado-Leonard and Miller, 1987).

The human *CYP17* gene is located on chromosome 10q24.3 (Fan et al., 1992). It consists of eight exons encoding 508 amino acids and is expressed in the adrenals and gonads. To date, more than 40 mutations in exons and introns of the *CYP17* gene have been reported to cause 17α-hydroxylase/17,20-lyase deficiency (Yanase, 1995; Auchus, 2001; Costa-Santos et al., 2004b,a). The disease is often characterized by the presence of homozygous or compound heterozygous mutations. Rare missense mutations of *CYP17* gene in the redox partner-binding domain cause isolated 17,20-lyase deficiency by disrupting interaction between P450c17 and its redox partner proteins (Geller et al., 1997). Herein, we report a female Taiwanese with 17α-hydroxylse deficiency. *CYP17* genes of this patient and five members of her family were analyzed by PCR-direct sequencing. Mutations in two alleles of this patient were confirmed by cloning and sequencing. Site-directed mutagenesis and in vitro functional analysis of this novel mutation [K494_V495 Del; R496L] were performed.

2. Materials and methods

2.1. Case report

The female patient, born in 1961, first visited the orthopedic department of our hospital because of her left femoral neck fracture caused by a car accident in 1987 at the age of 26. Hypertension and amenorrhea were noted during her visit, and endocrinologists were consulted to elucidate the problem. She had visited another hospital due to amenorrhea at the age of 20. Hypertension over 150–160/100–110 mmHg as well as hypokalemia were noticed at that time. Laparoscopy was performed previously and it revealed bilateral ovary atrophy and uterine agenesis. However, no definite diagnosis was made and no treatment was given at that time.

Physical examination revealed the following: body weight 46 kg, body length 158 cm, undeveloped breasts and external genitals, lack of axillary and pubic hair, no skin pigmentation, no hirsutism. Other findings were insignificant.

Chromosome study showed 46, XX. A series of clinical chemistry tests were performed. The results showed hypokalemia and mild hypernatremia (K^+: 2.1 mEq/L, Na^+: 149 mEq/L), suppressed renin activity, normal aldosterone, very high corticosterone, low cortisol and elevated ACTH. Progesterone was very high and 17OH-progesterone was low. Testosterone, estrogen and DHEA-S were low with high FSH and LH. The hormone concentrations were shown in Table 1. Image studies, abdominal sonography and CT were performed as well.

Table 1
Serum hormone concentrations in the patients with 17α-hydroxylase deficiency

	Patient	Reference range
Cortisol (nmol/L)	13.0	193.1–551.8
ACTH (pmol/L)	193.3	<15.8
Progesterone (nmol/L)	12.15	0.32–3.19
17OH-progesterone (nmol/L)	<0.30	0.61–9.08
Testosterone (nmol/L)	<0.007	0.485–2.982
Estradiol (pmole/L)	42.6	88.1–234.9
DHEA Sulfate (nmol/L)	<81.4	1080–10700
FSH (IU/L)	157.7	1–30
LH (IU/L)	239.3	1–30
Corticosterone (nmol/L)	695.53	2.89–14.43
PRA (ng/L s)	0.08	0.28–0.83
Aldosterone (pmol/L)	303.6	33.3–346.8

Right adrenal mass, which was about 3.2 cm × 2 cm, was noticed. Later in 1994, the right adrenal mass was surgically removed. The pathology report revealed adrenal cortical hyperplasia.

The clinical presentations and biochemical test results led to the diagnosis of 17α-hydroxylase deficiency. Glucocorticoid and sex hormone replacement therapy were in place. After many years of follow-up, we reviewed this case and performed a genetic analysis of the patient and her family members after obtaining informed consent. Her parents had no hypertension history at their young age. Her brother and two sisters were normotensive and had normal sexual development.

2.2. Hormone measurements

The patient's serum hormones were measured before steroid treatment, except that her corticosterone was tested 2 weeks after discontinuation of steroid in 2005, by the RIA, IRMA or EIA method with the following commercial kits—Cortisol, FSH and LH: Daiichi Pharmaceuticals, Tokyo, Japan; ACTH and plasma renin activity: DiaSorin Inc., Stillwater, MN; Testosterone, estradiol and progesterone: DPC, Los Angeles, CA; 17OH-progesterone: CIS BioInternational, France; DHEA-S: Dignostic Systems Laboratories Inc., Houston, TX; aldosterone: NEN, Boston, MA; and Corticosterone: Assay Designs Inc., Ann Arbor, MI.

2.3. DNA extraction and PCR

Genomic DNA was extracted from leukocytes of peripheral blood of the patient, her parents and three siblings using routine methods. All eight exons of the *CYP17* gene, including the flanking regions of introns, were amplified by PCR using eight pairs of primers as previously reported (Monno et al., 1997). The PCR products were confirmed by electrophoresis in 2% agarose gels.

2.4. Direct-sequence analysis

PCR products were purified by GFX™ using the Gel Band purification kit (Amersham Pharmacia, Uppsala, Sweden), and both strands were sequenced using the amplification primers as sequencing primers and Big-Dye Deoxy Terminator cycle sequencing reagents according to the manufacturer's instructions (ABI Biosystems Inc., Foster City, CA). Products of the sequencing reactions were purified by the ethanol/EDTA/sodium acetate precipitation method. Purified fragments were separated by capillary electrophoresis and detected via laser-induced fluorescence on an ABI PRISM 310 genetic analyzer (ABI Biosystems). Sequencing results were compared to the established human *CYP17* sequence (Accession No. NM_000102).

2.5. Cloning of mutant alleles and sequencing

The patient's PCR products were cloned into pGEM-T vector (Promega Inc., Madison, WI) and transformed to *E. coli* (DH5α) (Invitrogen, Carlsbad, CA), and eight random colonies were selected for direct sequencing.

2.6. Preparation of cDNA constructs

The full-length human *CYP17* cDNA was obtained by RT-PCR amplification of RNA isolated from the human adrenal carcinoma cell line NCI-H295 (ATCC CRL-10296, American Type Culture Collection, Manassas, VA) using a forward primer 5′-GCGAATTCAACCATGTGGGAGCTCGTGGCTCT-3′ and a reverse primer 5′-AATGGATCCTTAGGTGCTACCCTCAGCCT-3′. The cDNA for mutant *CYP17* [K494V495 Del; R496L] was generated by PCR-based mutagenesis with a reverse primer 5′-AATGGATCCTTAGGTGCTACC-CTCAGCCTGGGCTTCCCTCCAGGCCTGGA**G**~~CACCTT~~GATCTTCACTT-TGAA-3′ (start and stop codons are underlined; bolded "A" is the missensed site; deleted six base pairs are also shown). The PCR products were digested with *Eco*RI and *Bam*HI, and subcloned in-frame into the *Eco*RI and *Bam*HI sites of pFLAG-CMV2 (Sigma, St. Louis, MO). The resulting expression plasmids, pFLAG-h*CYP17* WT and pFLAG-h*CYP17* mt, were verified by DNA sequencing.