Case Report

Genomic Analysis of Two Siblings with 17α-Hydroxylase Deficiency and Hypertension

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17α-hydroxylase/17,20-lyase deficiency is an autosomal recessive disorder, which causes mineralcorticoid hypertension. Here we report two Japanese siblings, male and female, affected by 17α-hydroxylase/17,20-lyase deficiency. To clarify the molecular mechanism of the enzyme deficiency, we isolated the gene encoding 17α-hydroxylase/17,20-lyase (CYP17) by polymerase chain reaction (PCR) from these patients, and compared its nucleotide sequences with those of normal CYP17. We confirmed only one difference: a TTC of codon 53 or 54 was deleted in the exon 1 of CYP17 of the propositus, resulting in deletion of phenylalanine at either 53 or 54. Dot blot hybridization of the amplified DNA with allele-specific oligonucleotide probes showed that the two patients were homozygous and their parents were heterozygous for this mutation. The reduced activity of 17α-hydroxylase/17,20-lyase was probably caused by this mutation. (*Hypertens Res* 1994; 17: 143-147)

***Key Words*: 17α-hydroxylase deficiency, PCR, congenital adrenal hyperplasia, mineralocorticoid hypertension**

17α-hydroxylase/17,20-lyase activity is catalyzed by the microsomal cytochrome p450c17, which is encoded by a single gene formally termed CYP17 (*1*). Only a single enzyme catalyzing both 17α-hydroxylase and 17,20-lyase activities can be isolated from either the adrenals or gonads(*2, 3*). The single human CYP17 gene is expressed in the adrenals and gonads (*4*), but not in the placenta (*5*). 17α-hydroxylase deficiency impairs the synthesis of cortisol. The resulting low concentration of cortisol stimulates secretion of corticotropin, which causes adrenal growth (*6, 7*) and increases transcription of the CYP17 (*8, 9*) and p450scc (*10, 11, 12*), which are rate-limiting steps in steroidogenesis. Human 17α-hydroxylase deficiency is an autosomal recessive disorder and is one of the causes of congenital adrenal hyperplasia (*13*). In 17α-hydroxylase deficiency, adrenal 17 deoxysteroid, including deoxycorticosterone, corticosterone, 18-OH corticosterone, and aldosterone, upstream of the impaired enzyme step in the metabolic pathway, are increased in the tissues and blood, resulting in hypertension (*14*). The adrenals and testes of affected males also lack 17,20-lyase activity, and consequently fail to cleave the side chain of the C-21 steroids to produce the C-19 steroids, dehydroepiandrosterone and androstenedion. The fetal testes normally express 17α-hydroxylase early in gestation (*15*), and aldosterone is needed to develop external male genitalia (*16*). Human CYP17 cDNA has been isolated and sequenced (17), and CYP17 consisting of eight exons spanning 6.6 Kb has also been sequenced (*18, 19*).

Here we report two siblings with 17α-hydroxylase deficiency and identify the 3-base-pair deletion TTC in codon 53 or 54 in CYP17. We discuss the molecular mechanism of this mutation and the symptoms of 17α-hydroxylase/17, 20-lyase deficiency.

Case Report

Here we report two siblings with 17α-hydroxylase deficiency causing hypertension. The proposita was a 20-year-old Japanese male (II-2). He had been operated on for hypospadias and cryptorchidism as a child. His parents (I-1, I-2) were found to be not consanguineous. His father was from Miyako, in Sendai prefecture, and his mother was from Tokyo. Neither had apparent clinical abnormalities. Their blood pressures were normal. The patient's sister (II-2) was also diagnosed with 17α-hydroxylase deficiency by DNA analyses (Fig. 1). She menstruated, although irregularly. Her physical examination revealed no remarkable abnormalities. When the propositus visited our hospital, his blood pressure was 260/180 mmHg, and blood biochemical analysis showed hypokalemia (2.4 mEq/l). His sister's blood pressure was 172/108 mmHg, and mild hypokalemia was also found. Increased levels of ACTH, and 17-deoxysteroids including deoxycorticosterone (DOC) and aldosterone were confirmed in both patients,

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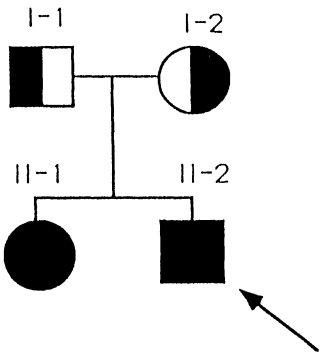

Fig. 1. *Pedigree of a Japanese family with 17α-hydroxylase deficiency. Solid and half-solid symbols represent homozygous and heterozygous individuals, respectively. Arrow indicates the proposita.*

suggesting low plasma renin hypertension. They also had decreased levels of testosterone. These data suggested a mild defect in 17α-hydroxylase activity (Table 1). After administration of dexamethasone, the blood pressure of the two patients normalized and they became normokalemic rapidly.

To analyze the molecular mechanism, heparinized blood samples were obtained from the patients and their family, and genomic DNA was prepared from leukocytes as previously described (*20*). Genomic DNA was completely digested with *Eco*RI (Takara, Kyoto, Japan), fractionate by electrophoresis in a 0.9% agarose gel, and transferred to a nylon membrane filter (Poll NY). Hybridization was done with the human CYP17 cDNA clone pCD17α-H (*17*), which was a gift of Dr. M.R. Watermann, Southwestern Medical School University of Texas (*21*). Southern blot analysis of *Eco*RI digested of genomic DNA from the patients showed 5.7- and 6.9-kb *Eco*RI fragments that were identical to those of the normal CYP17. (data not shown). These data suggested no remarkable deletion in the CYP17.

To further analyze the nucleotide level of the mutant CYP17, we cloned the CYP17 by the PCR method. One microgram of each DNA was amplified by PCR with Taq DNA polymerase (Promega, WI), and a set of two primers (Table 2) (*22*). One hundred picomoles of the PCR primers used corresponded to 20 base sequences of the 5′- and 3′-untranslated regions and introns so that all the protein coding regions and intron/exon splice junctions were amplified and cloned (*18, 19*). Nucleotide sequences were determined by the dideoxy methods as previously described (*23*), using kits purchased from Pharmacia. These nucleotide sequences exactly matched those of the normal CYP17 gene, with the exception of a deletion of a phenylalanine codon (TTC) either at amino acid position 53 or 54 of exon 1 (Fig. 2). As a result of this deletion, the mutant CYP17 is one amino acid shorter than normal CYP17.

To confirm that the mutation was present on both alleles and to eliminate the possibility that the mutation was generated during the cloning process, we hybridized the PCR-amplified products with two allele-specific oligonucleotide probes (*24*). The dot blot hybridization pattern verified the presence of the mutation in each individual in the pedigree (Fig. 3). As expected, the amplified DNA of the male patients and his sister hybridized only with the

Table 1. Laboratory data from a Japanese Family Affected by CYP17

Case	II-1	II-2	I-1	I-2	Normal range
Sexual abnormalities	none	hypopadias cryptorchidism	none	none	
Blood pressure (mmHg)	172/108 (132/72)	260/180 (140/80)	138/86	120/70	
K (mEq/l)	3.1 (3.0)	2.4 (4.1)	3.8	4.3	3.3-4.6
Plasma renin activity (ng/ml/h)	0.1 (0.3)	0.2 (0.2)	1.2	0.2	0.2-2.7
Aldosterone (ng/dl)	7.2 (2.4)	13.5 (4.0)	3.8	9.6	2-13
ACTH (pg/ml)	58 (11)	79 (10)	17	17	<60
DOG (ng/ml)	2.11 (0.28)	5.10	0.24	0.12	0.034-0.325
Cortisol (mg/dl)	3.7 (<1.0)	11.0 (<1.0)	8.3	11.7	5.6-21.3
Testosterone (ng/ml)	<0.1 (0.1)	0.9	3.2	0.2	4.0-14

Parenthesis in II-1 and II-2 indicate the value after administration of dexamethasone.

Table 2. Primers for the Polymerase Chain Reaction (PCR)

Sense primers	Exons amplified	Antisense primers
CTCTTCTACTCCACTGCTGT	1	TGAAGACCTGAACCAATCCCA
TGTAAGGGCAAGAGTGGGGT	2, 3	AGATTGGGGACAATGTCAGG
GGTGGAGTAGGAACTTCCAG	4	TGTGCCAGGTTCTCTGCTTG
TGGCAGGAGTGTCACAGATG	5, 6	CAAGCAGTGAATGCATCATGG
ATGAGGCTGAGCAAGGAAGG	7, 8	TGGACAGGGGCTGTGAGTTA

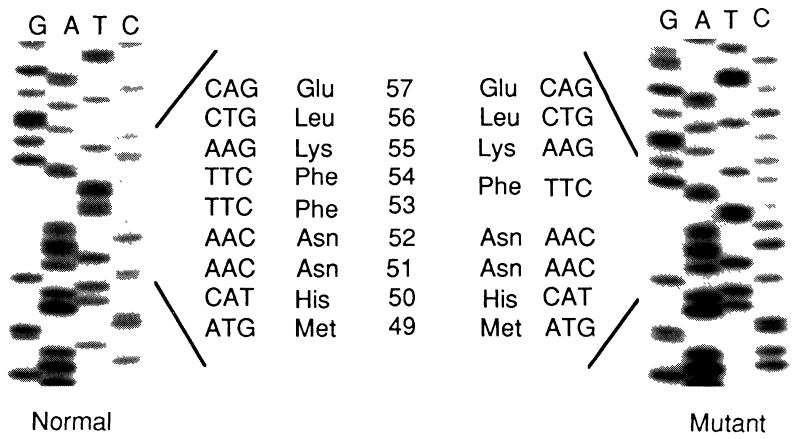

Fig. 2. *Sequence analysis of part of the exon 1 of CYP17 gene from the proposita and a normal individual. To clone the CYP17 gene, DNA isolated from the patients was amplified with a reaction mixture containing 67 mM Tris-HCl, pH 8.7, 19.6 mM $(NH_4)_2SO_4$, 0.02% gelatin, 0.45% Triton X-100, 250 μM each of dGTP, dATP, dTTP, and dCTP (Takara, Kyoto Japan), and 2.5 mM $MgCl_2$. Amplifications were done in a thermal cycler (Houei-Kagaku Japan) programmed as follows: 1) denaturation at 93 °C for 7 min; 2) addition of 4 unit of Taq polymerase followed by 30 cycles of denaturation at 93 °C for 2 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. Amplified fragments of each exon were purified from the gel and were cloned into pUC19 and sequenced (23). The TTC deletion at condon 53 or 54 causes the deletion of phenylalanine.*

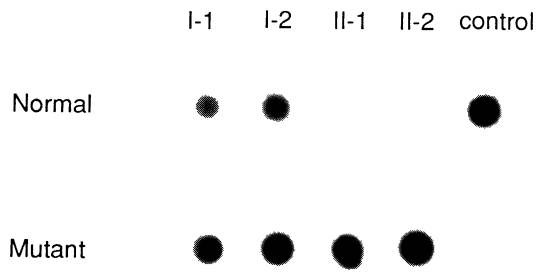

Fig. 3. *Allele-specific oligonucleotide hybridization analysis of genomic DNA amplified by the polymerase chain reaction method. Ten microliters of each of the PCR-amplified products from the family and a normal individual were mixed with 10 μl of 2N-NaOH and 80 μl of TE, and blotted directly onto a nylon membrane filter with a dot-blot apparatus (Bio-Rad CA). Each filter was rinsed with 2XSSC and hybridized with ^{32}P-end-labeled oligonucleotide proves as previously described (24). The sequences of the two probes were 5′-ACAACTTCTTCAAGCTGCA-G-3′ for the normal allelle and 5′-GCATAACAACTTC-AAGCTGC-3′ for the mutant allele. All oligonucleotides were synthesized on a DNA synthesizer (Millipore MA). Each amplified DNA derived from the proposita (II-2), his family members (II-1, I-1, I-2), and a normal individual (N) were hybridized to either the normal or mutant oligonucleotide probes.*

mutant probe, thereby indicating that they were homozygous for the mutation. Their parents were heterozygous for the mutation because hybridization signals were observed with both the normal and mutant probes. These results strongly suggested that the 3-base-pair deletion mutation reported here caused a 17α-hydroxylase deficiency.

Disccusion

In this study, a molecular defect was identified in two patients (one kindred) with 17α-hydroxylase deficiency by PCR. The nucleotide sequences of the coding region of the CYP17 were normal with the exception of a deletion of TTC at codon 53 or 54 in exon 1. According to amino acid sequence alignments of 17α-hydroxylase, the residue of the bacterial p450cam corressponding to the missing phenylalanine is not conserved but is thought to be located near the carboxyl-terminal end of the α-helix (*25*). The deletion of this phenylalanine at 53 or 54 of 17α-hydroxylase could change the α-helix to affect the secondary structure, causing a reduction in the enzyme activity.

Yanase *et al.* (*26, 27*) have reported 17α-hydroxylase deficiency caused by the same mutation. Their patient was an unrelated Japanese female, who lived in Nagasaki, which is very far from our patient's area of origin. She also had hypertension and hypokalemia and almost normal sexual differentiation, as did the female patient reported here. Our data confirm that the deletion of Phe 53 or 54 causes 17α-hydroxylase/17,20-lyase deficiency. Yanase *et al.* showed that the recombinant Δ53 or 54 mutant 17α-hydroxylase/17,20-lyase expressed in COS1 cells led to production of the same amount of immunodetectable protein as was found with transfection of normal CYP 17, but the 17α-hydroxylase activity of the mutant CYP17 was reduced to less than 37% and the 17,20-lyase activity was less than 8% of that observed with normal CYP17. These data suggest that the defect of the portion of 17α-hydroxylase containg either 53 and 54th phenylala-

nine might cause a reduction of 17,20-lyase activiy more profound than that of 17α-hydroxylase activity. The low 17,20-lyase activity from the mutation might decrease the C-19 steroids, dehydroepiandrosterone, androstenedione, and testosterone. But the abnomality in the external genitalia in the present male patient was not remarkable. There seemed to be some discrepancy between *in vitro* biochemical data and clinical symptoms in this man.

Yanase *et al.* also reviewed (*27*) seven other mutant alleles of CYP17 causing 17α-hydroxylase deficiency. Two cases were caused by nonsense mutations in codon 17 and 239 and one was caused by a 7-base deletion around codon 120. These mutations should produce truncated 17α-hydroxylase with no activity at all. These patients had symptomatic hypertension and female external genitalia in a genetic male. Other cases are caused by missense mutations in codon 342 (Pro-Thr) and codon 496 (Arg-Cys), a nonsense mutation in codon 461, and a 4-, base-pair duplication (CATC) around codon 480. These mutations were considered to produce minor change in the structure of 17α-hydroxylase, causing a reduction in the activity of the enzyme.

We assumed that the three mutant alleles of the 53 or 54 deletion of CYP17 reported here and by Yanase *et al.* were of independent origin, because the homes of patients were very far from each other. The direct repeated sequences (TTCTTC) surrounded by repetitive sequences are considered to be a hot spot for deletion mutations in replication. They might be explained by the slipped-mispairing model for the generation of deletions during DNA replication (*28*).

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