Combined 17 Alpha-Hydroxylase/17,20-Lyase Deficiency due to a Homozygous 25 BP Duplication (NT 4157-4181) at Exon 5 in the CYP17 Resulting in a Premature Stop Codon Predicted by Molecular Modeling

ABSTRACT

Combined 17alpha-hydroxylase/17,20-lyase deficiency is a rare, autosomal recessive form of congenital adrenal hyperplasia characterized by the coexistence of hypertension, caused by the hyperproduction of mineralocorticoid precursors and DSD in males and sexual infantilism in females, due to impaired production of sex hormones. Several *CYP17* mutations resulting in 17alpha-hydroxylase/17,20-lyase deficiency have been reported previously. In the present study, we described a novel CYP17 mutation in two Brazilian sisters with primary amenorrhea, 46,XY karyotype, high basal levels of progesterone (3.4-4.9 ng/mL) and hypokalemic hypertension born to consanguineous parents. After PCR and automatic sequencing of *CYP17* coding region, 25 bp duplication at exon 5 was found in the patients. This duplication started at codon 318 resulting in a premature stop codon at position 320 resulting in an ineffective and truncated protein and in accordance with the molecular modeling of P450c17. Therefore we expanded the repertoire of *CYP17* mutations describing the largest duplication found in this gene in both sisters, with a clinical phenotype of combined 17alpha-hydroxylase/17,20-lyase deficiency and emphasizes the importance of the P450c 17 molecular modeling to predict the functional effect of these mutations. **(Arq Bras Endocrinol Metab 2008; 52/8:1317-1320)**

Keywords: P450c17; Hypertension; Progesterone; Sexual infantilism; Ambiguous genitalia; Congenital adrenal hyperplasia; 46,XY DSD

RESUMO

Deficiência Combinada de 17 Alfa-Hidroxilase/17,20 Liase Devido a Duplicação de 25 PB (NT 4157-4181) no Éxon 5 do *CYP17* Resultando em Parada Prematura de Leitura Predita por Modelagem Molecular.

A deficiência combinada de 17 alfa-hidroxilase/17,20 liase é uma doença rara, de herança autossômica recessiva, causa de hiperplasia adrenal congênita caracterizada pela presença de hipertensão resultante do acúmulo de precursores mineralocorticóides, distúrbio da diferenciação sexual em homens e infantilismo sexual em mulheres devido à falha na produção de esteróides sexuais. Várias mutações no gene *CYP17* resultando em deficiência de 17 alfa-hidroxilase/17,20-liase têm sido descritas. No presente estudo, descrevemos uma nova mutação no *CYP17* em duas irmãs, nascidas de pais consangüíneos, com quadro de amenorréia primária, cariótipo 46,XY, dosagens basais elevadas de progesterona (3,4–4,9 ng/mL) e hipertensão hipocalêmica. Após PCR e seqüenciamento automático da região codificadora do *CYP17*, uma duplicação de 25 pb no exon 5 foi identificada nas pacientes. Esta duplicação inicia-se no códon 318 resultando em parada prematura de leitura no códon 320 gerando uma proteína truncada e inativa conforme predito pela modelagem molecular do P450c17. Com este achado, ampliamos o repertório de mutações do *CYP17* descrevendo a maior duplicação descrita até então neste gene em duas irmãs com fenótipo de deficiência combinada de 17 alfa-hidroxilase/17,20-liase e enfatizamos a importância da modelagem molecular do P450c 17 em predizer o efeito funcional destas mutações. **(Arq Bras Endocrinol Metab 2008; 52/8:1317-1320)**

Descritores: P450c17; Hipertensão; Progesterona; Infantilismo sexual; Genitália ambígua; Hiperplasia adrenal congênita; 46,XY DDS

clinical case report

Regina M. Martin
Paulo S. L. Oliveira
Elaine M. F. Costa
Ivo J. P. Arnhold
Berenice B. Mendonca

Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular LIM/42, Disciplina de Endocrinologia da Faculdade de Medicina da Universidade de São Paulo (RMM, EMFC, IJPA, BBM); Laboratório de Genética e Cardiologia Molecular, Instituto do Coração (InCor), Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (PSLO), São Paulo, SP, Brasil.

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INTRODUCTION

Cytochrome P450c17 (steroid 17-α-hydroxylase/17,20 lyase, EC 1.14.99.9) is a single microsomal enzyme that sequentially catalyzes two distinct reactions: the 17α-hydroxylation of steroids and the cleavage of the C17-20 carbon bond, converting C21 compounds to C19 steroids, essential for the production of glucocorticoids and sex steroids, respectively. P450c17 deficiency is an autosomal recessive disorder and a rare cause of congenital adrenal hyperplasia characterized by hypertension, hypokalemia and impaired production of sex hormones (1,2).

CASE REPORTS

We studied two 46,XY sisters who were raised as female and came to medical attention due to failure of breast development, absence of pubic hair and menarche. There was not complaint of weakness or cramps. Their parents are first degree cousins (Figure 1). Both patients had primary amenorrhea, absence of spontaneous breast development, absence of pubic hair, inguinal gonads, eunucoid habitus and blood hypertension (Table 1). Biochemical and hormonal measurements at diagnosis showed that both patients had normal levels of sodium, whereas potassium levels were borderline in the youngest sister and low in the oldest one (Tables 2 and 3).

Both cases had elevated basal and ACTH-stimulated progesterone levels and suppressed plasma rennin activity levels. On the other hand, basal 17OHP, Δ4-androstenedione, and DHEA levels were low and did not rise after ACTH stimulation. Serum cortisol was low with a subnormal elevation after ACTH stimulation. The P/17OHP and P/cortisol ratios (before or after ACTH stimulation) confirmed the 17α-hydroxylase defect (Table 4). Both patients had high gonadotropin levels, with very low testosterone and estradiol levels (Table 2). A human chorionic gonadotropin stimulation test performed before gonadectomy did not promote an androgen response indicating 17-20 lyase deficiency (Table 5). Treatment with low dexamethasone doses at night result in normalization of blood pressure, as well as of the progesterone and plasma rennin activity levels (Table 3).

Our aim was to analyze the *CYP17* coding region from these patients with combined 17α-hydroxylase/17,20-lyase deficiency phenotype and to predict their enzymatic activities using the human P450c17 molecular modeling proposed by Auchus and cols. (3,4).

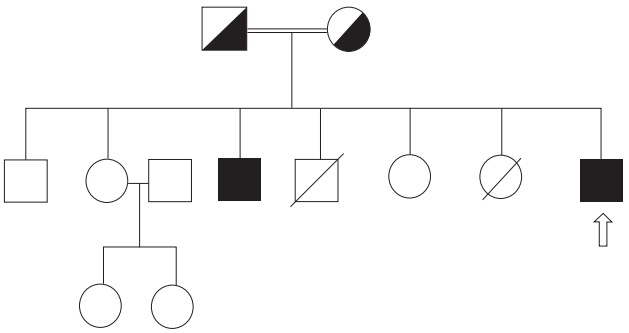

Figure 1. Family's pedigree; the arrow indicates the index case.

METHODS AND RESULTS

Genomic DNA was extracted from peripheral blood leukocytes from the patients, their mother and one normal control. PCR of the *CYP17* coding region was performed with intronic primers covering the flanking regions of each translated exon. After electrophoresis in agarosis gel, was possible to notice a subtle difference in the size of the *CYP17* exon 5 fragments. They were submitted to automatic sequencing reaction and 25 bp duplication in homozygous state from the patients samples could be revealed, whereas the mother's sequencing showed a heterozygous pattern (Figure 2). The others exons (1-4 and 6-8) of *CYP17* were also successfully amplified, submitted to sequencing reaction and no abnormalities were found.

DISCUSSION

Herein we report two sisters who presented the clinical and hormonal features typical of a combined form of 17 α-hydroxylase and 17,20-lyase deficiency (1,2). The hormonal profile was based on elevated progesterone levels (5) associated with significant reduction of cortisol and both adrenal and gonadal androgens. Besides, the disease was also confirmed by the molecular analysis of *CYP17* coding region from the patients. The result consisted of 25 bp duplication into exon 5 in homozygous state.

Although it is expected that this duplication would result in a premature stop codon at P450c17 (319 of 508 aa) – Figure 2C – and consequently producing an

Table 1. Clinical parameters at first medical examination.

Patient	Age (yr)	BP (cm Hg)	H (cm)	S (cm)	Axillary hair	Breast	Pubic hair	External genitalia	Gonads
1	16	14 x 11	149	154	-	T I	T I	Female	Inguinal
2	22	17 x 10	163	169	-	T IV*	T II	Female	Inguinal

BP = blood pressure; H = height; S = span; T = Puberty stage according Tanner. *Case 2 was previously treated with estrogen.

Table 2. Karyotype and basal hormone data of the patients.

Patient	Karyotype	LH (U/L)	FSH (U/L)	T (ng/dL)
1	46, XY	34.2	45.4	< 14
2	46, XY	17.9	19	< 14
NR (normal range)		1.4-9.2	1-12	200-950

Table 3. Laboratorial data before and during treatment.

Patient	K (mEq/L)	F (µg/dL)	ACTH (pg/mL)	Progesterone (ng/mL)	PRA (ng/ml/h)
Basal laboratorial data before treatment					
1	3.7	< 0.7	376	3.4	< 0.2
2	2.8	< 1.0	160	4.9	< 0.2
NR	3.5-5.5	6-28	<60	0.3-1.5	1.5-5.7
Laboratorial data during treatment with dexametasone 0.25 mg/day at night					
1	4.5	-	< 16.5	< 0.3	0.3
2	4.4	-	90	0.5	0.7

NR = normal range; K = potassium; F = cortisol; PRA = plasmatic rennin activity.

Table 4. Adrenal function before and after ACTH stimulation test.

Patient	Progesterone (ng/mL)		Cortisol (µg/dL)		17 OHP (ng/mL)		DHEA (ng/mL)		Δ4A (ng/mL)	
	B	P	B	P	B	P	B	P	B	P
1	3.4	3.7	< 0.7	< 0.7	0.3	0.3	0.2	0.2	< 0.2	< 0.2
2	4.9	5.3	< 1.0	< 1.0	0.8	0.9	0.7	0.8	0.2	0.2
NR	0.3-1.5	0.3-2.1	6-28	2.4-47	0.7-1.5	0.9-2.2	3-5.7	4.3-13	1.1-2	1.6-3.6

NR = ormal range; 17 OHP = 17-hydroxyprogesterone; Δ4A = androstenedione; B = basal; P = peak (after 250 µg of synthetic ACTH).

Table 5. Gonadal function before and after hCG stimulation test.

Patient	hCG (U/L)		17 OHP (ng/mL)		DHEA (ng/mL)		Δ4A (ng/mL)		T (ng/dL)	
	B	P*	B	P*	B	P*	B	P*	B	P*
1	< 5.0	29	0.3	0.2	0.2	0.2	< 0.2	< 0.2	< 14	< 14
2	< 5.0	128	0.8	0.7	0.7	0.9	0.2	0.2	< 14	< 14

17 OHP = 17-hydroxyprogesterone; Δ4A = androstenedione; T = testosterone; B = basal; P = peak. *48 h (case 1) and 72 h (case 2) after 5000 IU of hCG stimulation.

inactive truncated protein, we apply the molecular modeling of human P450c17 to characterize functional consequences of this novel *CYP17* mutation on enzyme function (3,4). It was clear that the heme-binding site, the substrate binding pocket, and the redox-partner site, all of which have been reported to be essential for catalytic activity, were lacking (Figure 3 E and F). Therefore, the P450c17 produced by our patient will have no activity, neither as 17α-hydroxylase nor as 17,20-lyase and it is in agreement with the phenotype described.

In conclusion we expanded the repertoire of *CYP17* mutations describing the largest duplication (in homozygous state) found in this gene in two 46,XY sisters with a phenotype of combined 17α-hydroxylase/17,20-lyase deficiency. This mutation causes a premature stop codon resulting in a truncated protein with dramatic loss of their enzymatic activities. The human P450c17 molecular modeling was an important tool to predict the loss of 17α-hydroxylase and 17,20 lyase activities with good correlation with patients' phenotype.

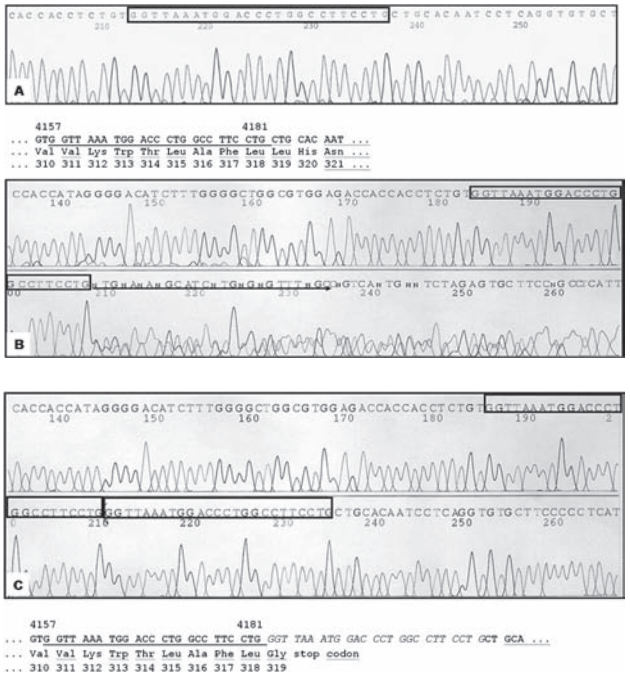

Figure 2. Impact of the 25 bp homozygous duplication of the *CYP17* exon 5 in the P450c17. (A) Fragment of *CYP17* exon 5 showing the 25 nt (nucleotides 4157-4181) inside the box at upper panel and underlined at botton. It represents the *wild type* pattern. (B) Duplication of 25 bp into *CYP17* exon 5 (underlined) in heterozygous state – *parent's* pattern. (C) 25 bp duplication *CYP17* exon 5 in homozygous state (second box at upper panel and in italic at botton) resulting in a premature stop codon at P450c17 enzyme.

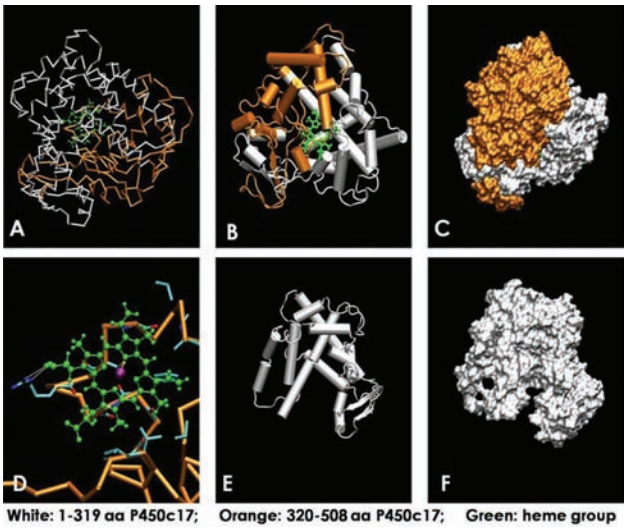

Figure 3. View of P450c17 wild-type: (A) backbone; (B) cartoon; (C) surface according to the human molecular modeling; (D) interactions between heme group and P450c17. View of the truncated P450c17 resulting by described mutation: (E) cartoon and (F) surface.

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Correspondence to:

Regina M. Martin
Hospital das Clínicas, Labaratório de Hormonios
Av. Dr. Eneas de Carvalho Aguiar, 155, PAMB, 2 andar, Bloco 6
05403-900 São Paulo SP
E-mail: reginamm@usp.br