Combined 17α-hydroxylase/17,20-lyase deficiency due to p.R96W mutation in the CYP17 gene in a Brazilian patient

Deficiência combinada de 17α-hidroxilase/17,20 liase devido à mutação p.R96W no gene *CYP17* em um paciente brasileiro

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SUMMARY

Congenital adrenal hyperplasia (CAH) resulting from 17α-hydroxylase/17,20-lyase deficiency is a rare autosomal recessive disease and the second most common form of CAH in Brazil. We describe the case of a Brazilian patient with CYP17 deficiency (17α-hydroxylase/17,20-lyase deficiency) caused by a homozygous p.R96W mutation on exon 1 of the *CYP17* gene, an unusual genotype in Brazilian patients with this form of CAH. The patient, raised as a normal female, sought medical care for lack of pubertal signs and primary amenorrhea at the age of 16 years. At evaluation, the presence of a 46,XY karyotype, hypertension and hypokalemia were observed. We emphasize the recognition of CYP17 deficiency in the differential diagnosis of cases of hypergonadotrophic hypogonadism and hypertension in young patients who need specific treatment for both situations. Arq Bras Endocrinol Metab. 2010;54(8):744-8

SUMÁRIO

A hiperplasia adrenal congênita (HAC), em razão da deficiência de 17α-hidroxilase/17,20-liase, é uma doença autossômica recessiva rara e a segunda causa mais comum de HAC no Brasil. Descrevemos o caso de um paciente brasileiro portador da deficiência 17α-hidroxilase/17,20-liase (CYP17) em homozigose para a mutação p.R96W no éxon 1 do gene da *CYP17A1*, uma mutação incomum entre os casos brasileiros descritos com essa forma de HAC. Esse paciente, criado como um indivíduo normal do sexo feminino, procurou atendimento por ausência de sinais puberais e amenorreia primária aos 16 anos de idade. Durante a avaliação, constataram-se um cariótipo 46,XY e a presença de hipertensão e hipocalemia. Enfatizamos o reconhecimento da deficiência da CYP17 dentre os possíveis diagnósticos em um paciente jovem com hipogonadismo hipergonadotrófico e hipertensão, os quais necessitam de tratamento particularizado para ambas as situações. Arq Bras Endocrinol Metab. 2010;54(8):744-8

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) resulting from 17α-hydroxylase/17,20-lyase deficiency is an uncommon autosomal recessive disease. The human 17α-hydroxylase (*CYP17*) gene is a single copy gene located on chromosome 10q24.3-q25 (1) that consists of 8 exons spanning 6,569 bases and encoding a protein of 508 amino acids (2). This protein is an enzyme expressed in the adrenal cortex and the gonads. Cytochrome P450 steroid 17α-hydroxylase (P450c17) catalyzes both the 17α-hydroxylation of pregnenolone and progesterone and also the 17,20-lyase reaction of 17α-hydroxypregnenolone/17α-hydroxyprogesterone to produce the C-19 steroid precursors of androgens and estrogens, dehydroepiandrosterone (DHEA) and androstenedione. Genetic abnormalities in the *CYP17* gene affect both adrenal and gonadal steroidogenesis.

The 17α-hydroxylase/17,20-lyase deficiency is associated with impaired production of cortisol and sex steroids leading to an elevation of plasma ACTH and overproduction of mineralocorticoids other than aldosterone resulting in hypertension, hypokalemia, and bilateral adrenal hyperplasia. In addition, the impaired production of sex steroids leads to abnormal sexual development. Therefore, this deficiency is clinically characterized by hypertension, hypokalemia, and sexual abnormalities such as 46,XY disorder of sex development (DSD) or sexual infantilism in 46,XX females (3-6).

In fact, 17α-hydroxylase/17,20-lyase deficiency seems to be the second most common form of CAH in Brazil (4,7). An founder effect may also contribute to the high prevalence of *CYP17* deficiency in Brazil, although the Brazilian population is among the most ethnically heterogeneous in the world (8). Previously, 30 Brazilian subjects with 17α-hvdroxylase deficiency from 24 kindred were reported and seven *CYP17* gene mutations were reported in this sample (9). Recently, a new mutation of 25 bp duplication at exon 5 of *CYP17* was described in two Brazilian sisters (46,XY kariotype), with the classical clinical presentation of this enzymatic defect and with P450c17 molecular modeling predicting the loss of both enzymatic activities of this protein (10).

Herein, we report the second case of 17α-hydroxylase/17,20-lyase deficiency in a Brazilian patient caused by a homozygous p.R96W mutation in the CYP17 gene and the fifth case in world literature.

CASE REPORT

A phenotypically female Brazilian patient presented at 16 years of age with lack of pubertal signs and primary amenorrhea. Her parents were consanguineous from Portuguese and Italian ancestry.

On physical examination her skin was darker than her parents. Recumbent blood pressure was elevated at 150 x 115 mmHg. Height was 151 cm and weight was 36,5 kg; arm span, inferior, and superior segments were 163 cm, 83 cm, and 68 cm, respectively. Tanner stage was B1P1, with an infantile female external genitalia and a blind vagina. Routine lab results showed normal sodium levels, BUN and creatinine; low serum potassium (3.3 mEq/L) and undetectable plasma renin activity (< 0.2 ng/mL/h); other results are shown in table 1. Her bone age was delayed (11 years), while the chronological age was 15 years/ 9 month-old (SD = 9.23 mo). Karyotype was 46,XY and uterus and gonads were not visualized on an abdominal-pelvic US. Patient's height was below calculated family target (172 cm for a boy although bone age was delayed).

Serum levels of 17α-OHP, DHEA-S, androstenedione and cortisol were low, whereas aldosterone, ACTH and progesterone were elevated. A cosyntropin stimulation test added evidence to the diagnosis revealing undetectable and unresponsive levels of cortisol and cortisone, 21-deoxycortisol (21DF), 11-deoxycortisol, and 17α-OHP, in presence of substantially elevated deoxycorticosterone (DOC) and corticosterone (Table 2).

Oral prednisolone was started at 5 mg/day without appropriate blood pressure and biochemical control; an increase to 7.5 mg/day normalized blood pressure levels and controlled steroid excess (Table 3). Subsequently, the patient was started on transdermic estradiol (25 mcg/day) with no interference with blood pressure control.

Table 1. Baseline biochemistry and hormone values in plasma

	Results	Normal range values
Cortisol 8h (µg/dL)	1.8	6.2-19.4
17α-OHP (ng/dL)	18	31-217
Estradiol (pg/mL)	< 5	7.5-42.5
LH (mUI/mL)	60.1	1.7-8.6
FSH (mUI/mL)	46.6	1.5-12.4
Total testosterone (ng/mL)	< 0.1	3.5-25
Progesterone (ng/mL)	6.14	0.2-1.4
DHEAS (µg/dL)	2	25-250
Androstenedione (ng/dL)	< 11	5-35
ACTH (pg/mL)	476	10-52
K (mEq/L)	3.2	3.5-5.1
Bicarbonate (mEq/L)	30	22-30
Aldosterone (ng/dL)	29	Supine: 2.9-16.2
PRA (ng/mL/h)	< 0.20	Upright: 0.98-4.18

17α-OHP: 17α-hydroxyprogesterone; PRA: plasma renin activity.

Table 2. Basal and ACTH–stimulated adrenal steroid values

Steroids	Baseline	Post-ACTH	Normal range values	
			Basal	Post-ACTH
Cortisol (µg/dL)	ND	ND	6-25	18-42
Cortisone (ng/dL)	ND	ND	800	3500
21Deoxycortisol (ng/dL)	26	68	0-40	< 156
11Deoxycortisol (ng/dL)	0.7	4.1	0-45	< 140
17α-OHP (ng/dL)	7	9	< 5	< 10
DOC (ng/dL)	527	612	4-12	12-61
Corticosterone (ng/dL)	18, 377	22, 030	0.1-0.5	1.7-4.8

17α-OHP: 17α-hydroxyprogesterone; DOC: deoxycorticosterone; ND: not done.

Table 3. Biochemistry and hormonal values after prednisone 7.5 mg/day

	Prednisolone 7.5 mg/day	Estradiol 25 µg/day	Normal range values
Cortisol 8h (µg/dL)	2.8	1.13	6.2-19.4
17α-OHP (pg/mL)	0.18	-	0.31-2.17
Progesterone (ng/mL)	0.28	1.31	0.2-1.4
DHEAS (µg/dL)	1.9	-	24.4-247
Androstenedione (ng/dL)	< 0.11	-	0.5-3.5
ACTH (pg/mL)	< 10	990	10-52
K (mEq/L)	5.6	4.5	3.5-5.1
Bicarbonate (mEq/L)	28	28	22-30
Aldosterone (ng/dL)	8.2	22.8	Supine: 2.9-16.2
PRA (ng/mL/h)	1.15	-	Upright: 0.98-4.18

17α-OHP: 17α-hydroxyprogesterone; PRA: plasma renin activity.

Genomic DNA was extracted from peripheral leukocytes and all eight exons of the *CYP17* gene were amplified by PCR (see below). The two most common mutations identified in Brazilian patients were not found in our patient; instead, an p.R96W (in exon 1) in homozygosis was detected. Neither her parents, nor her brother were hypertensive and their hormonal profile of serum progesterone, sex and adrenal steroids, gonadotropin, sodium and potassium were in the normal range.

DNA PREPARATION, PCR, AND SEQUENCING

DNA was extracted from peripheral leukocytes by a standardized salting out procedure (11). The 6.4-kb *CYP17* gene was amplified into 1-4 pieces from 0.5-1 µg genomic DNA using TaKaRa Ex Taq DNA polymerase (Takara Shuzo Co., Shiga, Japan) in 100 µL reactions using buffer and deoxy-NTPs provided by the manufacturer and 3% dimethylsulfoxide. To amplify 3- to 4-kb products, PCR parameters included 40 cycles of 3 min at 94°C, 1 min at 65°C, and 3 min at 70°C. Annealing time was increased to 1.5 min for amplification of the entire gene and the extension parameters were 72°C for 5.5 min. The final PCR products were precipitated with ethanol and purified on 1% agarose gels using the QIAEX II kit (Qiagen, Chatsworth, CA, USA). Amplicons were submitted to direct sequencing of the 8 exons and flanking intronic DNA by the dye termination method on a PE Applied Biosystems instrument (McDermott Center Sequencing Facility at University of Texas Southwestern Medical Center, Dallas, TX, USA). The mutations were identified by comparison with the GenBank sequence for CYP17 (accession no. M19489) using Mac Vector 6.5.3 (AccelrysCorp. San Diego, CA, USA) (12). Identified mutations were confirmed by sequencing the product of a second PCR amplification in the opposite direction (Figure 1). Parental DNA was not available for investigation.

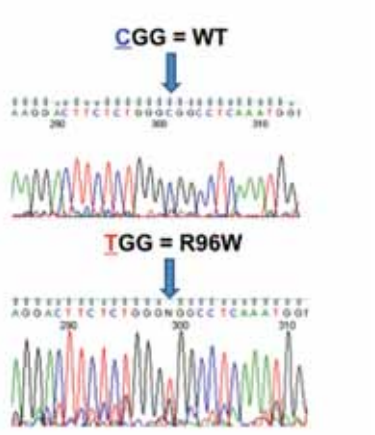

Figure 1. Electropherogram of p.R96W mutation with a transition C → T in codon Arg 96 (CGG) into a Trp (TGG) in exon 1.

DISCUSSION

The p.R96W mutation has been reported rarely in *CYP17*: in two 46,XY DSD French-Canadian siblings presenting with combined 17α-hydroxylase/17,20-lyase deficiency (12), an Italian patient (13) and also in another Brazilian patient (14). Our 16-year old patient sought treatment for delayed puberty and during clinical examination her blood pressure levels were elevated. Very often P450c17 deficiency remains undiagnosed until adolescence or early adulthood: overproduction of corticosterone substitutes for low cortisol production and prevents an adrenal crisis. Cortisol impairment results in secondary ACTH oversecretion and adrenal stimulation to produce large amounts of DOC which leads to clinical manifestations of mineralocorticoid excess: increased renal tubular resorption of sodium and kaliuresis, producing hypertension, hypokalemia and suppressed plasma renin activity (6). Thus, the adrenal zona glomerulosa does not receive renin stimulation for aldosterone synthesis, resulting in reduced aldosterone secretion (7). However, this is not the case in all *17OHD* patients, and some, as our patient, present serum hyperaldosteronism, the reason of which is still unclear and; possibilities include: a disorder of steroidogenesis and/or regulation or a cross-reaction between aldosterone and some other increased steroid(s) (5,14-15). The purpose of replacement glucocorticoid

therapy is to suppress the ACTH stimulation of zona fasciculata reducing the mineralocorticoid excess, and controlling hypertension and hypokalemia.

When combined 17α-OH and 17,20-lyase deficiencies are present, both the adrenal and gonadal production of sex steroids are impaired, leading to hypergonadotropic hypogonadism in both genetic sexes (7,16). Thus, replacement therapy with oral estrogen is necessary, running the risk that hypertension may be aggravated; this is possibly due to activation of estrogen receptors in the adrenal cortex, that may stimulate the altered mineralocorticoid's synthetic route in CYP17 deficient patients (17). Estrogen replacement was started in our patient with a transdermal 17β-estradiol formulation (25 µg/day). As expected, with the lack of first-pass hepatic metabolism and reduced GI enzymatic catabolism, the therapeutic effects could be achieved at lower peak doses and less impact on disease control. In fact, our patient's blood pressure did not change on this replacement scheme; however, elevation of ACTH, progesterone and aldosterone levels were observed together with a slight reduction of cortisol levels. This replacement schedule may also interact with steroidogenesis, but with no significant effect on blood pressure levels.

Structural modeling suggests that R96 lies within the flanking strand 2 of β-sheet 1, and the guanidine group of R96 appears to form hydrogen bonds with carbonyl groups of residues A113 and F114 (18). Removal of this positively charged group, which occurs in p.R96W mutation, appears to instabilize the protein by disrupting this interaction between the two domains, leading to complete enzyme inactivation (3).The severity of clinical disease tends to be milder with mutations that retain a partial catalytic activity (7,19), but the age of onset of hypertension, the degree of hypokalemia, and aldosterone production rate appear to vary, even among patients with the same *CYP17* mutations (10,15). Patients with partial 17α-hydroxylase deficiency can have ambiguous genitalia at birth (7,15,19). Recently a patient was described who carried both p.R96W and missense H373D mutation on the *CYP17* gene, showing that these mutations cause a dramatic loss of both enzymatic P450c17 activities (20). The missense p.R96W mutation causes a transition C→ T in codon Arg 96 (CGG) into a Trp (TGG) in exon 1 (12) (Figure 1), abolishing almost completely the activity of the mutant protein (3,20).

The p.R96W mutation can be explained by a founder effect, since our patient was from southern Brazil, an area traditionally under European migratory influence and whose population is more of a Caucasian ethnicity. The fact that founder effects can be manifested in a country with great ethnic heterogeneity also suggests a high coefficient of inbreeding in local areas.

Interestingly, CYP17 deficiency is the second most frequent form of CAH in Brazil (5,8), but to the best of our knowledge, the p.R96W mutation, has been identified just for the second time in Brazil. However, it is possible that the prevalence of this mutation may be higher than reported , especially in southern Brazil, a major German and Italian ethnic extract.

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