Six new cases confirm the clinical molecular profile of complete combined 17α-hydroxylase/ 17,20-lyase deficiency in Brazil

Seis novos casos confirmam o perfil clínico molecular de deficiência combinada de 17 alfa-hidroxilase/17,20-liase no Brasil

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SUMMARY

In 2004, Costa-Santos and cols. reported 24 patients from 19 Brazilian families with 17α-hydroxylase deficiency and showed that p.W406R and p.R362C corresponded to 50% and 32% of *CYP17A1* mutant alleles, respectively. The present report describes clinical and molecular data of six patients from three inbred Brazilian families with 17α-hydroxlyse deficiency. All patients had hypogonadism, amenorrhea and hypertension at diagnosis. Two sisters were found to be 46,XY with both gonads palpable in the inguinal region. All patients presented hypergonadotrophic hypogonadism, with high levels of ACTH (> 104 ng/mL), suppressed plasmatic renin activity, low levels of potassium (< 2.8 mEq/L) and elevated progesterone levels (> 4.4 ng/mL). Three of them, including two sisters, were homozygous for p.W406R mutation and the other three (two sisters and one cousin) were homozygous for p.R362C. The finding of p.W406R and p.R362C in the *CYP17A1* gene here reported in additional families, confirms them as the most frequent mutations causing complete combined 17α-hydroxylase/17,20-lyase deficiency in Brazilian patients. Arq Bras Endocrinol Metab. 2010;54(8):711-6

SUMÁRIO

Em 2004, segundo Costa-Santos e cols., p.W406R e p.R362C correspondiam a 50% e 32% dos alelos mutantes do gene *CYP17A1*, respectivamente, em 24 pacientes de 19 famílias brasileiras com deficiência da 17α-hidroxilase. Apresentamos os dados clínicos e moleculares de seis pacientes de três famílias consanguíneas brasileiras com deficiência da 17α-hidroxilase. Todas as pacientes apresentavam hipogonadismo, amenorreia e hipertensão ao diagnóstico. Duas irmãs tinham cariótipo 46,XY, ambas com gônadas palpáveis na região inguinal. Todas tinham hipogonadismo hipergonadotrófico, com nível aumentado de ACTH (> 104 ng/mL), atividade de renina plasmática suprimida, baixos níveis de potássio (< 2,8 mEq/L) e progesterona aumentada (> 4,4 ng/mL). Três delas, incluindo duas irmãs, apresentaram homozigose para a mutação p.W406R e as outras três (duas irmãs e uma prima) foram homozigotas para a mutação p.R362C. A recorrência das mutações p.W406R e p.R362C no gene *CYP17A1* aqui relatada em famílias adicionais confirma que essas são as mais frequentes causadoras do fenótipo completo da deficiência combinada de 17α-hidroxilase/17,20-liase em pacientes brasileiros. Arq Bras Endocrinol Metab. 2010;54(8):711-6

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Received on Jul/29/2010
Accepted on Nov/11/2010

INTRODUCTION

The microsomal cytochrome P450c17 is a single enzyme with two catalytic functions, 17α-hydroxylase and 17,20-lyase, which plays a crucial role in the biosynthesis of cortisol and sex steroids. Deficiency of P450c17 (17OHD) in both the adrenal cortex and gonads results

in impaired production of cortisol and sex hormones, leading to hypersecretion of ACTH and overproduction of mineralocorticoids (1,2).

P450c17 is encoded by the *CYP17A1* gene (OMIM 609300), which is located at 10q24.3 and contains eight exons (3-5). 17OHD is a rare form of congenital adrenal hyperplasia (CAH) with an estimated incidence of about 1:50,000 newborns (1), which represent 1% of all cases of CAH. Most reports describe isolated cases in consanguineous families (2), and approximately 50 different mutations in *CYP17A1* have been described, although some are more common and reoccur in certain ethnic groups. Most mutations have been seen to cause combined 17α-hydroxylase/17,20-lyase enzyme deficiency (1,2,6-17).

Typical features of complete 17OHD include hypertension, hypokalemia, and sexual infantilism in genotypic-phenotypic females, and as incomplete male sex differentiation with ambiguous or female genitalia, and also sexual infantilism in 46,XY subjects (1,2). Nevertheless, there is considerable variation in clinical and biochemical features of 17OHD (18), including the variant of isolated 17,20-lyase deficiency (19). The severity of the clinical disease tends to be milder with mutations that retain partial catalytic activity in assays using heterologous expression systems (1), but the age of hypertension onset, the degree of hypokalemia, and the aldosterone production rate appear to vary, even among patients with mutations that completely inactivate the enzyme (2).

In 2004, Costa-Santos and cols. (9) published clinical and molecular data of 24 patients with 17OHD from 19 kindreds from the Brazilian Congenital Adrenal Hyperplasia Multicenter Study Group and showed seven novel *CYP17* mutations. Two of them, p.W406R and p.R362C accounted for 50% and 32% of the mutant alleles (9).

We describe here clinical and molecular data of six additional Brazilian patients with 17OHD from three different kindreds.

CASE REPORTS

Clinical data and blood specimens of the patients and relatives were collected with approval by the appropriate institutional review board and a signed informed consent was obtained.

Tables 1 and 2 show clinical, biochemical and hormonal data from the six patients with 17OHD.

Patient 1 presented at age 15 because of absence of pubertal development signs (failure of breast development, absence of pubic hair and menarche). She had systolic hypertension (170/110 mmHg) since she was 14 years old. Her parents were second cousins. She had had four older sisters of unknown genetic sex who died at the ages of 5, 7, 9 and 13. The oldest sister, who died in her sleep, was also prepubertal and had hypertension.

Patients 2.1 and 2.2 are siblings born to consanguineous parents (first cousins). They were raised as girls and were examined at 17 and 18 years of age, respectively, because of lack of pubertal development (failure of breast development, absence of pubic hair and menarche). Both had hypertension since they were 12 years old (170/110 mmHg and 180/120 mmHg, respectively). Patient 2.2 had a stroke with permanent partial right facial paralysis by the time of hypertension diagnosis. They presented prepubertal female external genitalia with bilateral inguinal gonads but no uterus at ultrasound. Their karyotypes were both 46,XY.

Table 1. Clinical and biochemical features of 17OHD patients

Case	Age at diagnosis (yr)	Karyotype	External genitalia	Sex of rearing	BP (mmHg)	Na (mEq/L)	K (mEq/L)
1	15	46,XX	F	F	170/110	142	2.5
2.1	17	46,XY	F	F	170/110	141	2.6
2.2	18	46,XY	F	F	180/120	150	2.6
3.1	19	46,XX	F	F	180/110	149	2.7
3.2	18	46,XX	F	F	160/100	144	2.7
3.3	14	46,XX	F	F	160/100	142	2.8
Normal values						136 – 146	3.5 – 5.5

Table 2. Plasma basal hormonal values in 17OHD patients

	1	2.1	2.2	3.1	3.2	3.3	Normal values (adult female)
LH (IU/L)	31.2	26.1	67.3	30.5	28.9	25.2	1.9 – 12.5
FSH (IU/L)	50.0	52.5	81.7	80.6	75.7	88.2	1.5 – 8.0
ACTH (pg/mLl)	185	102	274	309	256	104	< 46
Cortisol (µg/dL)	2.4	2.2	2.6	2.8	2.6	2.6	5.0 – 25.0
PRA (ng/ml/h)	0.3	05	0.3	0.3	0.4	0.3	0.1 – 2.3
Progesterone (ng/mL)	4.4	5.0	4.9	4.6	6.6	8.4	0.1 – 1.4
17-OHprogesterone (ng/mL)	0.3	0.5	0.2	0.6	0.3	0.2	0.2 – 1.5
DHEA (ng/mL)	0.5	0.8	0.6	1.1	0.9	0.8	3.0 – 6.1
DHEA-S (µg/dl)	50	44	62	55	60	40	80 – 560
Androstenedione (ng/mL)	0.2	0.3	0.2	0.2	0.3	0.2	0.7 – 3.6
Testosterone (ng/dL)	0.06	0.06	0.08	0.06	0.06	0.08	0.06 – 0.85

Patients 3.1 and 3.2 are siblings born to consanguineous parents (first cousins) (Figure 1). They presented at the age of 18 and 19 because of absence of pubertal development signs (failure of breast development, absence of pubic hair and menarche) and hypertension (180/110 mmHg and 160/100 mmHg, respectively). They were prepubertal with female external genitalia. Their karyotypes were both 46,XX.

Patient 3.3 is a cousin of 3.1 and 3.2 and was also born to consanguineous parents (first cousins) (Figure 1). She was evaluated at the age of 14 because of delayed puberty (failure of breast development, absence of pubic hair and menarche). Hypertension was noticed during clinical evaluation (160/100 mmHg). Physical examination revealed absence of breast development, pubic and axillary hair with normal infantile female external genitalia. Her karyotype was 46,XX.

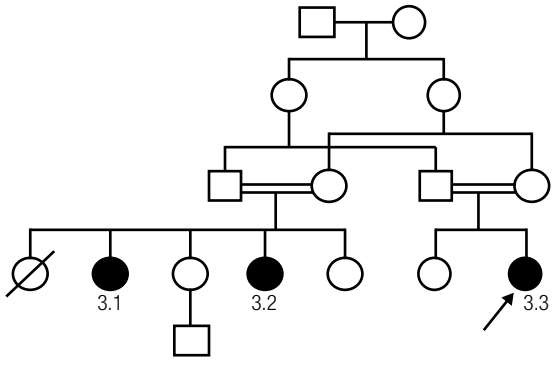

Figure 1. Pedigree of family 3.

Genomic DNA samples were obtained from peripheral blood by Proteinase K digestion and phenol/chloroform extraction following standard techniques. Primers for PCR amplifying the eight exons including exon-intron junctions of *CYP17A1* gene were designed using GeneRunner v3.0 free software. The amplified fragments were purified with Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI, USA) and both sense and antisense DNA strands were directly sequenced with Big Dye Terminator Cycle Sequencing Kit V3.1 Ready Reaction (ABI PRISM/PE Biosystems, Foster City, CA, USA). Sequencing reactions were electrophoresed in a ABI PRISM 3700 Automated DNA Sequencer capillary system according to the manufacturer's recommendations (ABI PRISM/PE Biosystems, Foster City, CA, USA). Sequences obtained were compared to the normal CYP17A1 genomic sequence (ENSG00000148795).

Patients 1, 2.1 and 2.2 showed the nucleotide change c.1388T>C in exon 7. The three patients were homozygous for the mutation that causes amino acid substitution p.W406R (Figure 2A). The *CYP17A1* sequence analysis of patients 3.1, 3.2 and 3.3 revealed a c.1256C>T homozygous transition in codon 362 located in exon 6. This nucleotide substitution leads to the p.R362C *missense* mutation (Figure 2B). Patients homozygous for p.R362C mutations were also homozygous for the less frequent allele of the following polymorphisms: rs743572 (5'UTR), rs6162 (exon 1), rs6163 (exon 1), rs743575 (intron 2), rs3740397 (intron 5), rs4919686 (intron 6), rs10883783 (intron 7) and a novel IVS-130del35bp. The deletion of 35 bp in

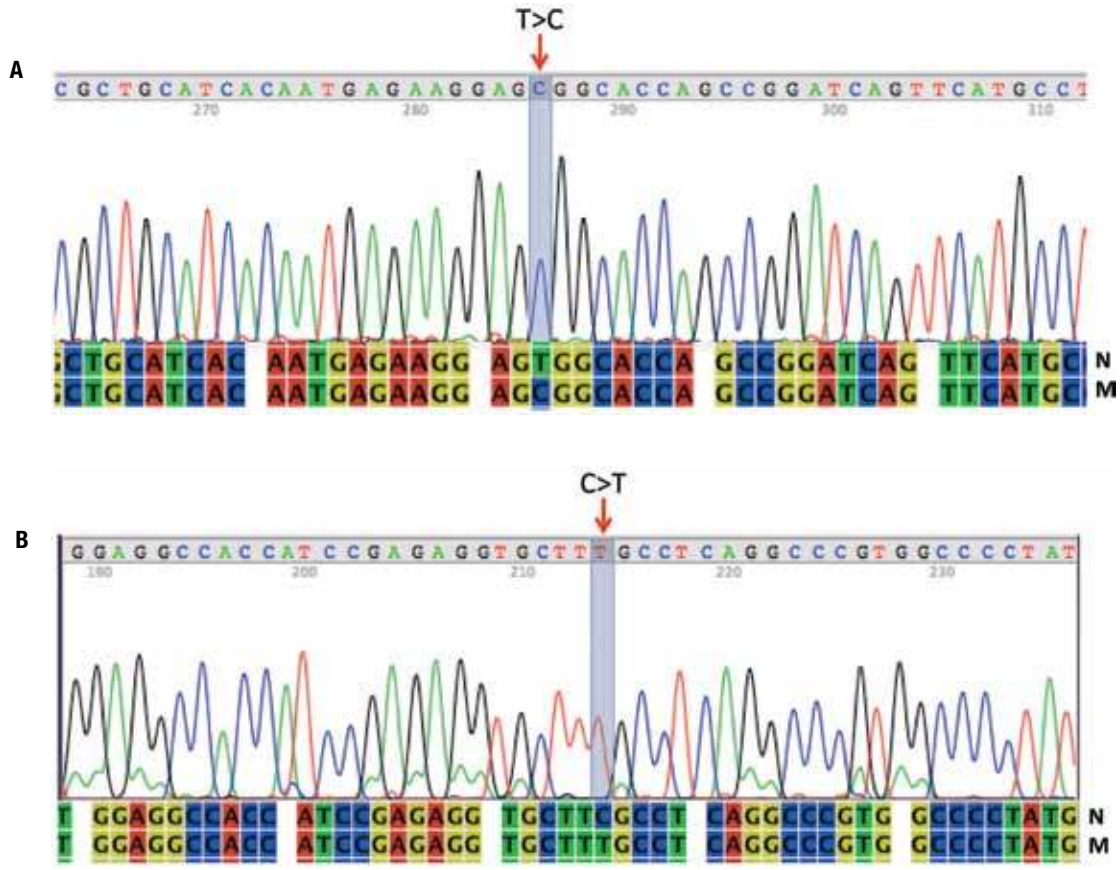

Figure 2. Eletropherograms showing parts of the *CYP17A1* gene sequences. **A**) Exon 7; the arrow denotes the c.1388T>C change causing the p.W406R mutation found in patients 1, 2.1 and 2.2. **B**) Exon 6; the arrow denotes the c.1256C>T change causing the p.R362C mutation found in patients 3.1, 3.2 and 3.3. N = normal sequence; M = mutant sequence.

intron 7 must be a frequent polymorphism in Brazilian population since it was found in 15.6% control alleles. Unfortunately, DNA samples of their parents were not available for analysis to confirm the segregation of mutations. However, patients have been considered homozygous for either p.W406R or p.R362C mutations without investigating microsatellites heterozygosis due to close consanguinity between parents and also due to the fact that *CYP17A1* gene deletions have never been reported.

DISCUSSION

The possibility of studying the *CYP17A1* gene of several patients affected by the rare 17α-hydroxylase/17,20-lyase deficiency is an effective tool to clarify the molecular mechanisms and genetic characteristics of the disease and also to gather more information about the structure-function relationship of this protein. P450c17 can be considered the qualitative regulator of steroidogenesis by determining which kind of steroids will be produced: mineralocorticoids in which P450c17 is absent, glucocorticoids when 17α-hydroxylation is active, and sex hormones when 17,20-cleavage takes place. Therefore, the knowledge concerning P450c17 will always be of great impact for understanding hypertension, adrenarche, puberty, and hyperandrogenism, with obvious implications in fertility.

Homozygous carriers of either p.W406R or p.R362C mutations have been described as presenting hypertension, hypokalemia, and sexual infantilism (9); a typical phenotype observed in complete combined 17α-hydroxylase/17,20-lyase deficiency (19). The six patients reported here, who were all born to consanguineous parents, also presented a complete combined 17α-hydroxylase/17,20-lyase deficiency phenotype, with moderate to severe hypertension, hypokalemia (< 2.8 mEq/L), elevated ACTH (> 104 ng/mL) and progesterone (> 4.4 ng/mL) with low levels of cortisol and 17OH-progesterone, and sexual infantilism (in both

46,XX and 46,XY subjects) with low levels of DHEA, DHEA-S, androstenedione and testosterone and high levels of LH and FSH. As showed by Martin and cols. (8), our data confirmed the importance of evaluating LH, FSH, ACTH, progesterone, and potassium and/or plasmatic renin activity to diagnose complete combined 17α-hydroxylase/17,20-lyase deficiency. A good genotype-phenotype correlation is therefore observed since p.W406R and p.R362C mutations showed no residual enzymatic activity in expression studies using COS-7 or HEK-293 cells and in yeast, indicating that the resulting phenotype for both mutations is the complete combined 17α-hydroxylase/17,20-lyase deficiency (9).

According to Costa-Santos and cols. (9), p.W406R and p.R306C account, respectively, for 50% and 32% of *CYP17A1* mutant alleles among Brazilian patients. The majority of affected individuals carrying p.W406R or p.R362C mutations are of Spanish or Portuguese descent, respectively (9). In contrast, no other mutation among almost 50 in the *CYP17* gene is related to patients of Spanish or Portuguese origin (1,2,6,7,10-12,14-17). Brazilians form one of the most heterogeneous populations in the world, as the result of more than five centuries of miscegenation from the four continents: America, Europe, Africa, and Asia. When the Portuguese arrived in Brazil in the year 1500, around 2.5 million South American Indians already lived in the country. The initial colonization involved almost exclusively Portuguese men, therefore, the first miscegenation occurred between European men, mainly Portuguese, and native Indian women. Between the years of 1500 and 1800 around half a million Portuguese men arrived in Brazil. Since the middle of the century to 1855, around four million African slaves came to Brazil. In 1808, Portuguese royalty moved from Portugal to Brazil and opened the ports to all nations. Between 1820 and 1975, around six million immigrants officially arrived in Brazil; 70% Portuguese and Italians, in equal numbers, followed by the Spanish, Germans, Syrians, Lebanese and Japanese (20). So, it is very difficult to define the correct ancestry of Brazilian patients, but the families included in the present study reported to have Spanish (patients 1, 2.1 and 2.2, all from Lagoa Santa – State of Minas Gerais – Brazil) and Portuguese (patients 3.1, 3.2 and 3.3, all from Jeremoabo – State of Bahia – Brazil) origins, confirming the data of Costa-Santos and cols. (9). The homozygosity for the less frequent allele in several polymorphisms found in patients 3.1, 3.2 and 3.3 might also indicate that this mutant allele corresponds to a rare *CYP17A1* haplotype introduced in Brazil by Europeans.

In conclusion, we confirm p.W406R and p.R362C mutations as causes of complete combined 17α-hydroxylase/17,20-lyase deficiency; a high genotype-phenotype correlation is observed for mutation carriers; and also they are the most frequent mutations in Brazilian patients, specially those with Spanish or Portuguese ancestry.

Acknowledgements: The authors would like to thank Dr. Márcio José da Silva from CBMEG sequencing facility for technical support.

Disclosure: no potential conflict of interest relevant to this article was reported.

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