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

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-hydroxylase 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.

SUMÁRIO

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 CYPI7A1 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 CYPI7A1 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 CYPI7 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**

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

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 \( \text{CYP17A1} \) 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 an 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 \( \text{CYP17A1} \) 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 \( \text{CYP17A1} \) 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

### Table 2. Plasma basal hormonal values in 17OHD patients

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

**Figure 1.** Pedigree of family 3.
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
of Brazilian patients, especially those with Spanish or Portuguese ancestry.

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REFERENCES


