Metabolic evidence for impaired 17\(\alpha\)-hydroxylase activity in a kindred bearing the E305G mutation for isolate 17,20-lyase activity

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Abstract

Context: The CYP17A1 gene encodes many enzymatic reactions including 17\(\alpha\)-hydroxylase and 17,20-lyase activities. Mutations that selectively ablate the 17,20-lyase activity, causing isolated 17,20-lyase deficiency, are exceedingly rare and may belong to the rarest of all disorders of steroidogenesis. We have previously reported an E305G mutation in the active site of CYP17A1 that apparently causes isolated 17,20-lyase deficiency. Expression studies suggested intact 17\(\alpha\)-hydroxylase activity which was at odds with subnormal tetracosactrin stimulated cortisol in the patients.

Objectives: To investigate the \(\textit{in vivo}\) activity of the adrenal enzymes, we used the metabolomics approach with urinary steroid profiling by gas chromatography–mass spectrometry.

Patients: Of the 11 subjects investigated, 6 patients in the kindred were found to be homozygous, 4 members were asymptomatic heterozygous, and 1 was homozygous for the wild-type allele.

Results: In the homozygous patients for E305G, both serum and urinary steroids showed a severe lack of androgens (C\(19\)-steroids) pointing to the absence of 17,20-lyase activities. Furthermore, precursor/product ratios of urinary steroid metabolites characterizing 17\(\alpha\)-hydroxylase activity showed variable decreases in 17\(\alpha\)-hydroxylase activities.

Conclusions: The results confirm the complete absence of 17,20-lyase activity \(\textit{in vivo}\), as in the \(\textit{in vitro}\) expression studies. On the other hand, \(\textit{in vivo}\) 17\(\alpha\)-hydroxylase activity was partially impaired. Thus, the \(\textit{in vivo}\) metabolic data seem to be more sensitive than the expression study and suggests that this mutation also impairs 17\(\alpha\)-hydroxylase activity.

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Introduction

The CYP17 gene encodes the cytochrome P450 enzyme that besides many other enzymatic reactions also catalyzes 17\(\alpha\)-hydroxylase and 17,20-lyase activities. Through its 17\(\alpha\)-hydroxylase activity, the enzyme converts progesterone and pregnenolone to 17-hydroxy-progesterone and 17-hydroxypregnenolone with similar affinity. For this reaction, the enzyme requires an intact steroid-binding domain and P450 oxidoreductase for electron transfer (1). For its lyase activity, the human enzyme converts mainly 17-hydroxypregnenolone to DHEA-S, whereas the conversion of 17-hydroxyprogesterone to androstenedione barely exists (2). For maximal lyase activity, the enzyme requires an interaction between the P450-oxidoreductase in the redox partner-binding site and the presence of cytochrome b\(_{5}\) (3, 4). The severity of the phenotype and the clinical presentation are related to the residual mutant enzyme activity. Mutations in the steroid-binding domain mainly result in combined 17\(\alpha\)-hydroxylase/lyase deficiency, whereas mutations in the redox partner domain have been found to result with isolated lyase deficiency (5).

Clinically, males with isolated 17,20-lyase deficiency have a female phenotype or ambiguous genitalia. Biochemically, this enzyme defect is characterized by low androgens and normal cortisol levels without hypertension.

In two members of this kindred, we have previously reported an E305G mutation in the active site of CYP17 that apparently causes isolated lyase deficiency (6) as suggested by expression studies. However, this result is at odds with subnormal-stimulated cortisol during tetracosactrin stimulation in some patients. We therefore used urinary steroid profiling by gas chromatography–mass spectrometry (GC–MS) to characterize the steroid metabolome of this unique kindred.
Patients and methods

Patients

The patients belonged to two families that are related by marital consanguinity; their pedigree is presented in Fig. 1. The first family consisted of seven children, with three clinically affected boys and a girl (Table 1).

The eldest son (IV6) was born after 38 weeks of normal pregnancy and delivery with a birth weight of 3700 g. On physical examination, he had micropenis of $1 	imes 0.5$ cm with perineal hypospadias, chordeae, and bifid scrotum, in which small testicles of 1 ml each were palpated. He was operated for hypospadias repair, until the age of nine when a pediatric endocrinologist saw him for the first time. At the age of ten, his height was 134.4 cm (50th percentile) and weight was 30.5 kg (75th percentile); the bone age was 9.5 years. He had a small penis (3 cm) with chordeae and scrotal testicles of 4 ml each. At the age of 11, he developed gynecomastia that reached Tanner stage B4 at the age of 14. At the age of 15, treatment with i.m. testosterone depot was begun and he started to shave at the age of 18. At the age of 19, his height was 179 cm (75th percentile), weight was 66.5 kg (75th percentile), his genitalia examination revealed a penis of length 7 cm and scrotal testicles of 20 ml. His endocrine evaluation is detailed in Table 2.

The second boy (IV7) was born after normal pregnancy and delivery with birth weight of 3700 g. He had micropenis without hypospadias or chordeae. A pediatric endocrinologist saw him first at the age of ten. His height was 133 cm (25th percentile) and weight was 28.5 kg (50th percentile). Genitalia examination revealed two mobile testicles of 2 ml each and a micropenis of 3 cm long. At the age of 14, his height was 179 cm (75th percentile), weight was 66.5 kg (75th percentile), his genitalia examination revealed a penis of length 7 cm and scrotal testicles of 20 ml. His endocrine evaluation is detailed in Table 2.

The third son of this nuclear family (IV12) was born after normal pregnancy and delivery with a birth weight of 4500 g. He had micropenis (1 cm) perineal hypospadias, chordeae, and bifid scrotum, in which small testicles of 1 ml each were palpated. Treatment with three monthly injections of 25 mg i.m. testosterone depot doubled his penile length. He was operated for correction of hypospadias at the age of 11 months.

All the daughters in this family have normal female genitalia. They were evaluated by tetracosactrin stimulation and urinary steroid profiling. The affected girl (IV10) was found to have low tetracosactrin stimulation-stimulated cortisol level. On physical examination at age ten, she had normal clitoris and vaginal opening, she grew along the 25th percentile and her weight was on the 10th percentile.

The second family consisted of four boys and a girl. One daughter died after delivery for an unknown cause. Subject IV5 was referred during puberty for evaluation of micropenis and gynecomastia. On physical examination at the age of 14, he had gynecomastia (Tanner stage B4), normal testes (15 ml each), and a small (3.4 cm) phallus with sparse pubic hair. His history revealed an uncomplicated pregnancy and delivery. Severe hypospadias with chordeae, bifid scrotum, and micropenis were noted at birth. The hypospadias was surgically repaired and the penile growth was treated with three testosterone enanthate injections of 25 mg/month during infancy. All his other brothers had normal genitalia.

In order to explore the suspected defect in his sister (IV2), a tetracosactrin stimulation was performed and an abnormal response was detected at the age of 18 (Table 2). Her history revealed normal pregnancy and delivery, normal growth, normal breast development, and irregular menses. On physical examination, normal female genitalia with sparse pubic hair were observed. Ultrasonography showed a big cyst in each ovary of 6.5
and 5.3 cm. None of the patients in the kindred had hypertension or hypokalemia.

**Genotyping**

Written informed consent was obtained from parents. Genomic DNA was extracted from peripheral leukocytes using standard protocol. Exon 5 of \textit{CYP17A1} was amplified with Promega Go-Taq in a 25 μl reaction mix according to the manufacturer’s protocol using \textit{CYP17A-F} and \textit{CYP17A-R} as primers (5-ATCTCTAG-TCAAGGAGAAG-3 and 5-CAGGCCTAGTCTTCCTG-CAC-3 respectively). The PCR parameters included 30 cycles of 30 s at 94 °C, 30 s at 60 °C, and extension 35 s at 72 °C, giving a 410 bp PCR product. The presence of mutation E305G was sought by the elimination of an EcoR31I cleavage site, resulting in a single 410 bp product, rather than the wild-type 240 and 170 bands as shown in Fig. 2.

**Table 1** Clinical data of the children in the kindred.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Year of birth</th>
<th>Sex</th>
<th>Birth weight (kg)</th>
<th>Karyotype</th>
<th>Genotype</th>
<th>Clinical presentation at birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV 2</td>
<td>1985</td>
<td>F</td>
<td></td>
<td>46XX</td>
<td>Homozygous</td>
<td>Normal female genitalia</td>
</tr>
<tr>
<td>IV 5</td>
<td>1984</td>
<td>M</td>
<td></td>
<td>46XY</td>
<td>Homozygous</td>
<td>Severe micropenis, perineal hypospadias, chordae, bifid scrotum, 1 ml testicles</td>
</tr>
<tr>
<td>IV 6</td>
<td>1985</td>
<td>M</td>
<td>3.6</td>
<td>46XY</td>
<td>Homozygous</td>
<td>Severe micropenis, perineal hypospadias, chordae, bifid scrotum, 1 ml testicles</td>
</tr>
<tr>
<td>IV 7</td>
<td>1988</td>
<td>M</td>
<td>3.7</td>
<td>46XY</td>
<td>Homozygous</td>
<td>Severe micropenis, perineal hypospadias, chordae, bifid scrotum, 1 ml testicles</td>
</tr>
<tr>
<td>IV 8</td>
<td>1989</td>
<td>F</td>
<td>3.0</td>
<td>46XX</td>
<td>Heterozygous</td>
<td>Normal female genitalia</td>
</tr>
<tr>
<td>IV 9</td>
<td>1991</td>
<td>F</td>
<td>3.6</td>
<td>46XX</td>
<td>Heterozygous</td>
<td>Normal female genitalia</td>
</tr>
<tr>
<td>IV 10</td>
<td>1993</td>
<td>F</td>
<td>3.8</td>
<td>46XX</td>
<td>Homozygous</td>
<td>Normal female genitalia</td>
</tr>
<tr>
<td>IV 11</td>
<td>1999</td>
<td>F</td>
<td>3.8</td>
<td>46XX</td>
<td>WT</td>
<td>Normal female genitalia</td>
</tr>
<tr>
<td>IV 12</td>
<td>2000</td>
<td>M</td>
<td>4.5</td>
<td>46XY</td>
<td>Homozygous</td>
<td>Micropenis, without hypospadias, normal scrotum, with palpable testicles</td>
</tr>
</tbody>
</table>

**Table 2** Endocrine evaluation by tetracosactrin stimulation tests in five patients (basal and peak) and hCG test in two patients (peak).

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Normal</th>
<th>60 min tetracosactrin-stimulation test</th>
<th>Normal tetracosactrin stimulation test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone nmol/l</td>
<td>3.9–9.9</td>
<td>4.5–0.5</td>
<td>10.1–12.9</td>
<td>1800–4500</td>
</tr>
<tr>
<td>Corticosterone pmol/l</td>
<td>401</td>
<td>90–600</td>
<td>2022</td>
<td></td>
</tr>
<tr>
<td>Aldosterone pg/ml</td>
<td>57–111</td>
<td>40–300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma renin activity ng/ml per h</td>
<td>0.4–1.8</td>
<td>&lt;5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 hydroxyprogrenolone nmol/l</td>
<td>1.2–8</td>
<td>40–5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 hydroxyprogesterone nmol/l</td>
<td>2.2–20.9</td>
<td>&lt;15</td>
<td>1.9–16</td>
<td>&lt;30</td>
</tr>
<tr>
<td>11 Deoxycortisol nmol/l</td>
<td>8.1–8.4</td>
<td>&lt;23</td>
<td>6.1–9.6</td>
<td>&lt;23</td>
</tr>
<tr>
<td>Cortisol nmol/l</td>
<td>67–192</td>
<td>&gt;90</td>
<td>200–383</td>
<td>&gt;540</td>
</tr>
<tr>
<td>DHEA-S μmol/l</td>
<td>0.2–0.63</td>
<td>0.95–11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androstendione nmol/l</td>
<td>&lt;0.35</td>
<td>&gt;0.35</td>
<td>&lt;0.35</td>
<td>&gt;0.35</td>
</tr>
<tr>
<td>Testosterone nmol/l</td>
<td>&lt;0.65</td>
<td>F 2.9–0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M 0.98–39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen pmol/l</td>
<td>309</td>
<td>&gt;73 F</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Serum steroids**

Serum cortisol, estradiol, and DHEA-S were measured by a solid phase chemiluminescent enzyme immunoassay (CLEIA: DPC Los Angeles, CA, USA). The 11-deoxycortisol was measured by RIA Double Antibody (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA). The 17-hydroxyprogesterone was measured by RIA-coated tubes (CIS Bio international, Bagnols/Coze, France). Testosterone and progesterone were measured by competition principle electrochemiluminescent (ECLA; Elecsys 2010, F.Hoffmann-La Roche, Basel, Switzerland).

**Urinary steroids**

Urinary steroid profiles were determined from spot urine specimens using quantitative data produced by GC–MS analysis according to the method described elsewhere (7). Free and conjugated steroids were extracted by solid
phase extraction and the conjugates were enzymatically hydrolyzed. The hydrolyzed steroids were again recovered by solid phase extraction, derivatized, and analyzed by GC–MS.

**Results**

Eleven family members were genotyped for the E305G mutation in the CYP17 gene. Five were found to be homozygote for the mutation, five heterozygous, and one was homozygous for the wild type. The genotype/phenotype relationship is summarized in Table 1.

**Serum steroids**

The adrenal steroids profile, summarized in Table 2, demonstrated low C19-steroids. DHEA-S, androstenedione, and testosterone were very low or undetectable in affected patients. The 17-hydroxysteroids were normal or high. The patients had low basal serum cortisol levels that ranged between 67 and 192 nmol/l, with a mean of 130±45 nmol/l (normal >90 nmol/l). They also had insufficient response of cortisol to tetraacosactrin stimulation. (250 μg Synacten, Novartis, France) with peak levels that ranged between 200 and 383 nmol/l, with a mean of 293±75 nmol/l (normal >540 nmol/l). Progesterone and estrogen levels were normal according to their age. All patients had normal blood pressure, normal aldosterone, and normal plasma renin activity.

Two patients underwent human chorionic gonadotrophin (HCG) stimulation test (5000 units) at ages 11 and 12; DHEA-S and androstenedione remained undetectable; testosterone levels increased in one patient from <0.69 to 2.5 nmol/l (normal >6.5 nmol/l) and did not respond in the other patient.

**Urinary steroids**

A comparison between the urinary steroid metabolomes of a homozygote patient and a healthy subject as assessed by GC–MS is given in Fig. 3. Our diagnostic parameters for assessing enzymatic activity are based on precursor/product ratios, as presented in Table 3. All affected subjects had highly elevated metabolic ratios of corticosterone metabolites/C19-steroids (B–M/An + Et) reflecting impaired global activity of the enzymatic system 17α-hydroxylase/17,20-lyase. The highest values were found for the ratio of metabolites within the Δ5-pathway (P5T/(A5-3β,17β)) indicating no conversion of 17-hydroxyprogrenolone to DHEA-S. The 17α-hydroxylase activity was impaired in all patients, though not to the same extent as lyase activity. Practically, all heterozygous relatives had pathological markers of 17,20-lyase activity, though not as expressed in homozygote. Slightly impaired activity of the 17α-hydroxylase activity could only be found in two heterozygous individuals. No patient had significant impairment of 21-hydroxylase activity (Tables 3 and 4).

**Discussion**

Mutations that selectively ablate the 17,20-lyase activity, causing isolated 17,20-lyase deficiency, are exceedingly rare and may belong to the rarest of all disorders of steroidogenesis. The 17,20-lyase deficiency has so far been described only in a few cases where mutations were found at the redox partner site, R347H, R347C, and R358Q (8). In each of these sites, mutations were directly responsible for a negligible activity (<1–5%) of the 17,20-lyase in the in vitro expression studies. However, even in these cases where the mutation directly affects the 17,20-lyase activity site, 17α-hydroxylase activity was affected with residual activities of 44.1–65, 13.6, and 65% respectively.

The combination of low cortisol, testosterone, and DHEA-S in our patients suggested a defect in CYP17, which is required for the conversion of C21-steroids to C19-steroids in both the adrenal glands and gonads. Yet 17-hydroxysteroids were normal, suggesting selective impairment of lyase activity.

Amplification and direct sequencing of all CYP17 gene exons in our patients showed a GAG-to-GGG missense mutation at codon 305 in exon 5. This mutation substitutes a glycine for the highly conserved glutamate at this position, which resides within the active site pocket. The Glu305 is found in a region of the I-helix, corresponding to cytochrome P450 substrate recognition site (13); substitution of these residues may uniquely influence the binding of various substrates.

Kinetic studies that were conducted in yeast and transfected human embryonic kidney (HEK)-293 cells expressing the mutant E305G demonstrated that in vitro lyase activity was seriously affected by this mutation; DHEA-S production was barely detectable, even when cytochrome b5 was added to the incubation. However, the conversion of 17-hydroxyprogesterone to androstenedione was found to be slightly more rapid compared with wild-type CYP17. The conclusion from the expression studies was that the mutation E305G selectively impairs 17,20-lyase activity for DHEA-S synthesis while an increased capacity to form androstenedione from 17-hydroxyprogesterone was found.

Assessment of the patients’ steroid metabolomes by urinary GC–MS steroid profiling allowed us to estimate the relative activities of 17α-hydroxylase and 17,20-lyase. The 17,20-lyase activity was absent in the homozygote patients as it has been found in the in vitro expression studies. However, unlike the expression studies, 17α-hydroxylase activity was found to be partially reduced. The discrepancy between the results in the in vitro expression studies and those of the urine studies are a key finding of this study. Possible explanations remain speculative; they include different protein stability, different membrane phospholipid
The presence of mutation E305G was sought by the elimination of an EcoR31I cleavage site, resulting in a single 410 bp product, rather than the wild-type 240 bp and 170 bp bands in nine subjects of the kindred. *represent non-specific bands.

**Figure 2**

**Figure 3** GC–MS urinary steroid profiles (scan runs) in (A) a healthy male (20 years) and (B) a patient with isolated 17,20-lyase deficiency (23 years). In the healthy male's profile, the presence of the cortisol metabolites (17-hydroxylated C21-steroids) tetrahydrocortisone (THE), tetrahydrocortisol (THF), and 5α-tetrahydrocortisol (αTHF) indicate normal 17α-hydroxylase activity. The presence of the androgen metabolites (C19-steroids) androsterone (An) and etiocholanolone (Et) reflects normal 17/20-lyase activity. In contrast, the patient's profile is dominated by corticosterone metabolites such as tetrahydrocorticosterone (THB), 5α-tetrahydrocorticosterone (αTHB), and tetrahydro-11-dehydrocorticosterone (THA) which, together with a reduction of cortisol metabolites, reflect impaired 17α-hydroxylase activity. The lack of 17-oxygenated C19-steroids (An and Et) proves the absence of 17,20-lyase activity. Internal standards are indicated by Roman numbers (I, 5α-androstane-3α,17α-diol; II, stigmasterol; and III, 5-cholestone-3β-ol-butyrate).
The recently newly described form of congenital adrenal hyperplasia, P450 oxidoreductase deficiency (14), can be confused with 17-hydroxylase deficiency. In P450 oxidoreductase deficiency, the activity of not only 17α-hydroxylase but also 21-hydroxylase is impaired (15). Therefore, key metabolites of 21-hydroxylase deficiency are elevated in the urinary steroid profiles of patients with P450 oxidoreductase deficiency as well. However, our metabolic data show that 21-hydroxylation was not affected in our patients and therefore 21-hydroxylase activity is not the reason for the blunted cortisol response to the tetracosactrin-stimulation test. Other theoretical possibilities for the blunted cortisol response are low 11β-HSD1 activity or low corticosteroid-binding globulin.

The results shed some light onto the role of the Δ5 steroid pathways in penile development. In these patients, the main production pathway of testosterone composition and differences in P450 oxidoreductase content. Urinary steroid profiles of heterozygotes for the E305G mutation inconsistently found slightly impaired 17α-hydroxylase and consistently impaired lyase activity. A clear differentiation between heterozygotes and unaffected relatives was not possible.

Table 3 Urinary steroid metabolome of homozygous for E305G mutation and age and gender-matched controls (upper range limits in parentheses). Diagnostic ratios of precursor/product metabolites. Pathological values are in bold (22, 23).

<table>
<thead>
<tr>
<th>Subject symbol</th>
<th>IV 2</th>
<th>IV 5</th>
<th>IV 6</th>
<th>IV 7</th>
<th>IV 10</th>
<th>VI 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Global 17α-hydroxylase/lyase activity</td>
<td>50.1 (0.3)</td>
<td>10.7 (0.5)</td>
<td>22.7 (0.3)</td>
<td>67.7 (0.6)</td>
<td>66.6 (1.7)</td>
<td>442.3 (2.6)</td>
</tr>
<tr>
<td>Δ5-pathway</td>
<td>0.37 (0.29)</td>
<td>3.18 (0.11)</td>
<td>3.43 (0.29)</td>
<td>0.86 (0.46)</td>
<td>6.29 (1.21)</td>
<td>10.37 (0.93)</td>
</tr>
<tr>
<td>Δ4-pathway</td>
<td>0.54 (0.14)</td>
<td>0.83 (1.33)</td>
<td>0.64 (0.14)</td>
<td>0.53 (0.32)</td>
<td>19.13 (0.34)</td>
<td>1.25 (0.07)</td>
</tr>
<tr>
<td>B/M An + Et</td>
<td>2.83 (0.17)</td>
<td>4.18 (0.30)</td>
<td>7.63 (0.17)</td>
<td>10.24 (0.29)</td>
<td>10.04 (0.43)</td>
<td>8.50 (0.34)</td>
</tr>
<tr>
<td>21-hydroxylase activity</td>
<td>0.02 (0.05)</td>
<td>0.01 (0.01)</td>
<td>0.05 (0.05)</td>
<td>0.02 (0.02)</td>
<td>0.09 (0.02)</td>
<td>0.04 (0.15)</td>
</tr>
</tbody>
</table>

B-M, sum of corticosterone metabolites; An, androsterone; Et, etiocolanolone; P5D, pregnenediol; P5T-17α, 11-oxopregnanetriol; a-CL, α-cortolone.

Table 4 Urinary steroid metabolome of heterozygous for E305G mutation and one unaffected subject IV11, age and gender matched controls (upper range limits in parentheses). Diagnostic ratios of precursor/product metabolites. Pathological values are in bold. (22, 23)
via Δ5 is severely interrupted and DHEA-S production is barely detected. The testis can generate C19-steroids androsterone and D via 158 androstanediol from 17α-oxosteroid and androstaneol from 17α-hydroxyprogesterone without the intermediary of androstenedione and testosterone. The brisk metabolism of 5α-pregnan-3α,17α-diol-20-one to androsterone by CYP17 explains how, when 5α-reductases are expressed, the testis generates C19-steroids androsterone and androstaneol from 17α-hydroxyprogesterone without the intermediate of androstenedione and testosterone. It is possible that this pathway was also affected in our subjects investigated, the androsterone/etiocholanolone ratio was not elevated and thus not indicative of an active back door pathway (16).

The fact that our patients presented at birth with microgenitalia, and hypospadias and not with female genitalia indicates that they had sufficient testosterone and dihydrotestosterone levels during embryogenesis for the development of a penis; however, the low levels of testosterone and dihydrotestosterone during the third trimester diminished penis development and resulted in micropenis with hypospadias. It turns out that androstenedione formation from 17α-hydroxyprogesterone via the minor Δ4-steroid pathway and 5α-reductases is not sufficient for complete formation of the male phenotype in humans.

One explanation for the testicles growth during puberty in the affected members IV5, IV6, and IV7 can relate to their high follicle-stimulating hormone (FSH) levels stimulating the growth of testicular tubules (17). The gynecomastia in these patients can be due to the low levels of androgens that resolved partially during testosterone replacement treatment.

Ultrasonad studies revealed cystic ovaries in one female. It confirms previous reports of the same in subjects with R347C mutation (18–21). Elevated FSH and luteinizing hormone levels, secondary to estrogen deficiency, might have caused ovarian stimulation.

The present study was initiated to understand the discrepancy between expression studies and the blunted serum cortisol response to tetracosactrin-stimulation test in these patients. Thus, the in vivo metabolic data seem to be more sensitive than the expression study, as we previously reported, and lead to the conclusion that this mutation does not solely affect 17,20-lyase but also impair 17α-hydroxylase activity.

Acknowledgements

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