**Short Report**


Steroid 11β-hydroxylase deficiency is the second most common cause of congenital adrenal hyperplasia, resulting in virilization, glucocorticoid deficiency and hypertension. The 11β-hydroxylase enzyme is encoded by the CYP11B1 gene and mutations in this gene are responsible for this disease. The aim of this study was to characterize mutations in the CYP11B1 gene and to determine their frequencies in a cohort of Tunisian patients. The molecular genetic analysis was performed by direct nucleotide sequencing of the CYP11B1 gene in 15 unrelated Tunisian patients suffering from classical 11β-hydroxylase deficiency. Only two mutations were detected in homozygous state in the CYP11B1 gene of all patients, the p.Q356X in exon 6 (26.6%) and the novel p.G379V in exon 7 with large prevalence (73.3%). This is the first report of screening for mutations of CYP11B1 gene in the Tunisian population and even in the Arab population.

**Key words:** 11β-hydroxylase – congenital adrenal hyperplasia – CYP11B1 – mutation

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Received 20 January 2010, revised and accepted for publication 9 February 2010

Congenital adrenal hyperplasia (CAH) is one of the most common inherited endocrine disorders following an autosomal recessive trait (1, 2). Whereas the majority of cases of CAH are caused by steroid 21-hydroxylase deficiency (21-OHD) (3), steroid 11β-OHD (OMIM_202010) accounts for 5–8% of cases (4) and has the frequency of 1/200,000 in the general Caucasian population (5).

The 11β-OHD is divided into classical and non-classical forms according to the clinical manifestations. Deficiency in 11β-hydroxylase causes a decrease in cortisol secretion, a subsequent elevation of plasma levels of adrenocorticotropic hormone (ACTH), and accumulation of steroid precursors. Many of these precursors are shunted into the androgen synthesis pathway, leading to hyperandrogenism. In classical form, typical signs of androgen excess include masculinization of female external genitalia and precocious pseudopuberty in both sexes, resulting in short adult stature. In about two thirds of patients, hypertension can be diagnosed because of an accumulation of 11-deoxycorticosterone and its metabolites (5, 6). Female patients with non-classic 11β-OHD are born with normal genitalia and present with signs of androgen excess as male patients.

The CYP11B1 gene, which encodes the 11β-hydroxylase enzyme, comprises nine exons. It
11β-Hydroxylase deficiency in Tunisia

is located on chromosome 8q22, approximately 40 kb from the highly homologous CYP11B2 gene encoding for the aldosterone synthase (7).

More than 50 different mutations have been described in the CYP11B1 gene, which are distributed over the entire coding region, whereas a cluster is reported around exons 2, 6, 7, and 8, suggestive of mutational hot spots (8–10). These mutations have been identified from diverse ethnic backgrounds and most are family-specific mutations.

Here, we report the molecular genetic analysis of the CYP11B1 gene in 15 unrelated Tunisian families with patients suffering from 11β-OHD. Our aims are to identify mutations in the CYP11B1 gene and to determine their frequency in this cohort. This is the first countrywide study presenting molecular data from Tunisian patients with 11β-OHD.

Patients and methods

Study subjects

Molecular analysis concerned 15 unrelated Tunisian CAH patients who were referred to the Department of Congenital and Hereditary Disorders of the Charles Nicolle Hospital in Tunis. All patients (10 females and 5 males) are suffering from classical 11β-OHD, including accelerated bone age, precocious pseudopuberty, and ambiguous genitalia in females (Table 1). All families were unrelated and originated from five different regions of the country. Consanguinity was present in 12 families. Blood samples were obtained from all patients and from their parents if available. An additional group of 53 normal Tunisian subjects was recruited as controls. Informed consent for mutation analysis was obtained from all patients or their parents and normal subjects.

Molecular genetic studies

Genomic DNA was prepared from peripheral blood leukocytes using standard procedures. The molecular genetic analysis was performed after polymerase chain reaction (PCR) amplification by direct DNA sequencing of CYP11B1 gene as described previously (11,12). Once a mutation was identified for a patient, segregation of the corresponding mutation was studied in both parents, every time available. The CYP11B1 genomic DNA (gDNA) (GenBank NG_007954.1) numbering corresponds to +1 of the A of the ATG translation initiation codon. PCR reaction was performed in 1.5 mM Mg, using 1 U Taq polymerase (Invitrogen, San Diego, CA) and buffer supplied by the manufacturer. Reactions were performed in a thermocycler (Applied Biosystems 9700, Foster City, CA) under the following conditions: initial denaturation at 95°C for 5 min, then 30 cycles of 95°C denaturing for 30 s, 60°C annealing for 30 s, 72°C extension for 30 s, and a final step of 10 min at 72°C. PCR products were purified utilizing Wizard® SV gel and PCR Clean-Up System (Promega Corporation, Madison, WI). Sequencing was performed utilizing the BigDye® Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems) as described by manufacturer except for only

Table 1. Clinical, hormonal, and molecular data in the 15 Tunisian patients with 11β-hydroxylase deficiency

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Phenotype (Prader stage)</th>
<th>Blood pressure (mm Hg)</th>
<th>Basal serum S (nmol/l)a</th>
<th>Consanguinity</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>7 days</td>
<td>AG (III)</td>
<td>–</td>
<td>ND</td>
<td>0</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>22 days</td>
<td>AG (III)</td>
<td>–</td>
<td>ND</td>
<td>0</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>6 years</td>
<td>AG (IV)</td>
<td>145/100</td>
<td>Inc</td>
<td>1/16</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>5 years</td>
<td>AG (IV)</td>
<td>140/100</td>
<td>150.0</td>
<td>1/32</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>3 years</td>
<td>AG (IV)</td>
<td>150/110</td>
<td>Inc</td>
<td>1/16</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>2 days</td>
<td>AG (III)</td>
<td>–</td>
<td>Inc</td>
<td>1/16</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>5 years</td>
<td>PPP</td>
<td>170/110</td>
<td>156.6</td>
<td>1/16</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>7 years</td>
<td>PPP</td>
<td>160/110</td>
<td>213.0</td>
<td>1/16</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>3 years</td>
<td>PPP</td>
<td>135/90</td>
<td>185.0</td>
<td>1/64</td>
<td>p.G379V/p.G379V</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>15 days</td>
<td>AG (IV)</td>
<td>–</td>
<td>208.8</td>
<td>1/32</td>
<td>p.Q356X/p.Q356X</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>8 days</td>
<td>AG (III)</td>
<td>–</td>
<td>250.0</td>
<td>1/16</td>
<td>p.Q356X/p.Q356X</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>6 years</td>
<td>AG (IV)</td>
<td>?</td>
<td>Inc</td>
<td>1/16</td>
<td>p.Q356X/p.Q356X</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>12 years</td>
<td>PPP</td>
<td>150/100</td>
<td>Inc</td>
<td>1/16</td>
<td>p.Q356X/p.Q356X</td>
</tr>
</tbody>
</table>

AG, ambiguous genitalia; F, female; Inc, increased; M, male; ND, not determined; PPP, precocious pseudopuberty.

aBasal 11-deoxycortisol (S) level, normal values (0.58–4.48 nmol/l).
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2 μl of Ready mix and 3 μl of 5× sequencing buffer in a 20-μl reaction. The samples were electrophoresed on an automated ABI PRISM 3130 sequencer and analysed with the ABI Seq-Scape 2.5 software (PE Applied Biosystems).

Results

In all of the 15 Tunisian patients suffering from classical 11β-OHD, mutations within the coding region of the CYP11B1 gene were identified by sequencing analysis in homozygous state. We found only two modification patterns in the 30 unrelated alleles. The DNA sequence of the entire coding sequence and the promoter region was determined, and no other mutation was found. In 11 patients (22 of 30 alleles; 73.3%), we identified a new missense mutation at codon 379 in exon 7, consisting of a G to T transition (g.4516G>T) in a homozygous form, which results in the substitution of glycine to valine (p.G379V) (Fig. 1). Sequencing the opposite strand of the PCR products confirmed these findings. In six families, DNA samples are available from both parents and both are heterozygous for this single nucleotide change. No p.G379V was found among 53 healthy controls. The G379 amino acid sequence alignment showed that the glycine is highly conserved among different species. In the four remaining patients (8 of 30 alleles; 26.6%), we identified a previously described non-sense mutation (p.Q356X) in exon 6 in a homozygous state (9). As expected for consanguineous families, both parents are heterozygous for p.Q356X mutation.

Discussion

This study is the first report about the distribution of mutations causing 11β-OHD in the Tunisian population and even in the Arab population. The rate of consanguinity in our series is 80%, no other population reported in literature has such a high rate. Consanguineous families were unrelated and originated from five different regions of Tunisia. In this study, we describe a novel missense mutation p.G379V in the exon 7 of CYP11B1 gene.

![Fig. 1](image1.png)

Fig. 1. The novel missense p.G379V mutation of the CYP11B1 gene at homozygous status. (a) Patient and (b) normal sequence.
This mutation was found in 11 of 15 (73.3%) unrelated patients in a homozygous form. All 10 affected females (46,XX) had a severe phenotype with ambiguous genitalia, grade III–IV on the scale of Prader. In all five affected males (46,XY), the diagnosis has been delayed and done during 2 and 4 years of life in the presence of pseudo-precocious puberty often associated with hypertension. The novel p.G379V mutation should prevent synthesis of a functional enzyme. As this glycine located between K-helix and B1-3 sheet constitutes the structure of the putative active site very close to valine 378, one of many hydrophobic amino acids; this mutation changing glycine to valine should modify this active site. The p.G379V mutation is carried by 73.3% of unrelated haplotype and appears very specific of Tunisian population, because it has never been reported previously. Further studies should be done in North Africa and Arab population to confirm its specificity. In a similar example, Tunisian patients with 21-OHD present a high frequency of p.Q318X mutation in CYP21A2 gene, with 37% in contrast to 0.5–13.8% described in other populations (13).

In conclusion, we detected only two mutations in Tunisian patients having 11β-OHD. Based on the present data, the screening of these two mutations could confirm rapidly the diagnosis of 11β-OHD and prenatal diagnosis should be informative in most cases.

Conflict of interest

We declare that we have no conflict for each author.

References