Establishment of clinical and lab algorithms for the identification carriers of mutations in CYP21A2

**background**

Bi-allelic mutations of CYP21A2 encoding 21-hydroxylase are the most frequent cause of congenital adrenal hyperplasia (CAH). Non-classical CAH (NCAH) or even just hyperandrogenism may be caused by mild or single heterozygous mutations of CYP21A2. These mutations are associated either with elevated basal or ACTH-stimulated levels of 17-hydroxyprogesterone (17OHP) in blood.

**objective**

The objective of this study was to identify the most suitable of six different test algorithms and the determination of appropriate cut-off levels for that test to recognize patients with NCCAH and carriers of clinically relevant mutations in CYP21A2. Test algorithms were composed around 17OHP measurements.

**patients**

Between July 2006 and July 2015 ACTH-tests were conducted in 768 children and adolescents (Age ≤ 20 y) suspected to have NCCAH. Inclusion criteria were premature pubarche with accelerated bone age, hyperandrogenemia, hirsutism, or menstrual irregularities.

**methods**

The compared test algorithms were:

- 17OHPBasal: Basal 17OHP levels
- 17OHP30: Stimulated 17OHP levels 30 min after ACTH was given
- 17OHP60: Stimulated 17OHP levels 60 min after ACTH was given
- delta17OHP: Difference between basal and stimulated 17OHP levels
- sum 17OHP: Sum of basal and stimulated 17OHP levels
- ratio 17OHP: Quotient of stimulated 17OHP divided by basal 17OHP

Receiver operating characteristics (ROC) were plotted and the most suitable test was identified by the greatest area under the curve (AUC). For 371 patients with delta17OHP ≥ 2.5 μg/l genomic blood DNA of the subjects CYP21A2 gene was analyzed by DNA-sequencing and MLPA. Cut-off levels were determined from ROC by pythagorean theorem.

**results**

Among the six tested algorithms, delta17OHP revealed the highest AUC with respect to identifying both patients carrying single (0.762) or bi-allelic (0.920) mutations. Basal 17OHP values by themselves proved weak in identifying single heterozygous and bi-allelic carriers.

<table>
<thead>
<tr>
<th>Test Variable</th>
<th>Area under the curve (AUC)</th>
<th>Asymptotic Significance</th>
<th>Area under the curve (AUC)</th>
<th>Asymptotic Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>17OHPBasal</td>
<td>0.573</td>
<td>0.039</td>
<td>0.811</td>
<td>0.001</td>
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<tr>
<td>17OHP30</td>
<td>0.756</td>
<td>0.000</td>
<td>0.908</td>
<td>0.000</td>
</tr>
<tr>
<td>17OHP60</td>
<td>0.758</td>
<td>0.000</td>
<td>0.900</td>
<td>0.000</td>
</tr>
<tr>
<td>Delta 17OHP</td>
<td>0.762</td>
<td>0.000</td>
<td>0.920</td>
<td>0.000</td>
</tr>
<tr>
<td>Sum 17OHP</td>
<td>0.728</td>
<td>0.000</td>
<td>0.888</td>
<td>0.000</td>
</tr>
<tr>
<td>Ratio 17OHP</td>
<td>0.599</td>
<td>0.005</td>
<td>0.609</td>
<td>0.238</td>
</tr>
</tbody>
</table>

**conclusion**

Not only bi-allelic but also single heterozygous mutations of CYP21A2 may be associated with clinical signs such as premature pubarche and hyperandrogenism. Our results suggest calculating the difference between stimulated 17OHP values 60 minutes after ACTH was given and basal 17OHP before ACTH was given. Genotyping of CYP21A2 is proposed in patients with values exceeding 3.4 μg/l diff.17OHP. Bi-allelic mutations, i.e. CAH or NCCAH are associated with diff.17OHP >10 μg/l.