the different physical properties of the amino acids are a major reason for the changes in enzymatic activity, the short distance of a variation to an active site of the enzyme can also be causative. In 2001, Belkina et al studied the three-dimensional structures of the CYP11B1 and CYP11B2 genes and proposed the I helix of 11β-hydroxylase as the putative active site. Since the variant p.Arg43Gln is located within the N-term and not within the helix I, Barr et al assumed that this specific variant disturbs a substrate recognition site or influences the flexibility of the enzyme and disrupts the substrate-active site combination. It should also be noted that p.Arg43Gln changes a basic arginine amino acid into a neutral polar glutamine amino acid, suggesting functional protein impairment.

Patient 2 was found to be a symptomatic carrier of the novel p.Val484Asp mutation and it can be assumed that this mutation contributes to the partial enzyme impairment. The specific mutation exchanges a non-polar valine hydrophobic amino acid for a positively charged polar aspartic acid amino acid at codon region 484, causing PCOS. Neighboring codons Tyr485 and Ile488 are parts of the active CYP11B1 gene site. It can therefore be predicted that mutation p.Val484Asp affects the local conformation and disturbs the efficiency of 11β-hydroxylase thereby contributing to the patient’s phenotype. Interestingly, in silico mutation analyses that were performed initially using Provean software predicted a deleterious effect of the p.Val484Asp alteration on CYP11B1 function (Table 2).

<table>
<thead>
<tr>
<th>Variant</th>
<th>PROVEAN score</th>
<th>Prediction (cutoff=-2.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p.Val484Asp</td>
<td>-3.777</td>
<td>Deleterious</td>
</tr>
</tbody>
</table>

Genetic screening for CYP11B1 mutations in 75 Greek-Cypriot female patients with mild symptoms of hyperandrogenemia included: Group A with 37 females who displayed a premature adrenarche phenotype, and group B with 38 females with polycystic ovary syndrome all of whom had been previously examined for mutations in the CYP21A2 gene. Of the 75 patients, 23 were found to be heterozygotes, whereas the remaining 52 had no CYP21A2 mutations. Finding from our previous study that demonstrating the frequency of the underlying genetic CYP21A2 defects in the Cypriot population to be one of highest ever reported and with an allelic frequency of 1:10 corroborated the observed heterozygotes of the present study. Several studies have demonstrated CYP11B1 gene defects to be rare, therefore, it is not surprising that of the patients tested in the present study only three NC-CAH patients were identified with CYP11B1 gene mutations.

The detection of only two carriers of 11β-hydroxylase deficiency mutations among female patients with mild hyperandrogenemia is consistent with other studies and demonstrates that CYP11B1 gene mutations account for a small minority of cases in females with hyperandrogenism. The novel p.Val484Asp mutation was found to cause a distortion of the surrounding beta sheet and also to result indirectly in destabilization of the binding cavity that occurs on the opposite face of the structural elements, leading to partial impairment of the enzymatic activity. Reporting novel mutations and even combinations are of great importance as such information can enable a clearer diagnosis and contributes to a better treatment modality. Understanding the extent of cortisol synthesis impairment caused by specific mutations but also due to a combination of mutations is essential for correct diagnosis, prognosis and treatment. In this report we examine the deleterious effect of a novel CYP11B1 mutation and, in addition, how heterozygosity in CYP21A2 as well as cases with no identified mutations could lead to a NC-CAH phenotype similar to that observed in the most prevalent type of the disorder with two identified mutations.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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