Copy number determination of CYP21A2 gene supplements the molecular biological analysis of Hungarian patients with 21-hydroxylase deficiency

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Background

Congenital adrenal hyperplasia (CAH) is a rare (prevalence 1:15000), autosomal recessive disorder caused by 21-hydroxylase deficiency in 95% of all cases. This disorder is related to the mutation of CYP21A2 gene that is located in a multiallelic complex called RCCX module showing tandem copy number variation. Molecular genetic analysis of genes located in such region is frequently difficult but the accurate diagnosis of patients suspected with CAH requires a complex molecular analysis of this region.

Objective

1. To analyze the most common mutations of the CYP21A2 gene
2. To determine the copy number of the CYP21A1P and A2 genes in our patients clinically diagnosed with CAH

Results

In the classical form among the examined 140 chromosomes we found deletions in 39,3%, the 12 splice in 27,8% and in 42,1% one of the 5 frequent mutation was detected. By using complex molecular biological analysis 58 of 70 (82,8%) cases were resolved.

In the non-classical cases deletions in 20,7%, 12 splice mutation in 4,8% and in 64,6% cases one of the 5 most frequent mutations was detected. Totally in 31 of 41 patients (75, 6%) could the genotype correctly determined.

Determination of copy number variations is an accurate and helpful method in molecular diagnosis of CAH. It may lead to a faster diagnosis for CAH suspected patients. The lacking mutations suggest that other methods including whole sequencing of the CYP21A2 gene and analysis of large deletions by MLPA should also be included into the molecular biological workup.

Patients and Methods

We studied 111 clinically diagnosed CAH patients (45 salt wasting, 25 simple virilising and 41 non classical/late onset). The most frequent mutations Δ8bpE3, P30L, IVS2-13A>C-G, I172N, R356W, Q318X) of the CYP21A2 were screened by allele-specific PCR. The copy number of CYP21A2 and its pseudogene was measured by real-time quantitative PCR.