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

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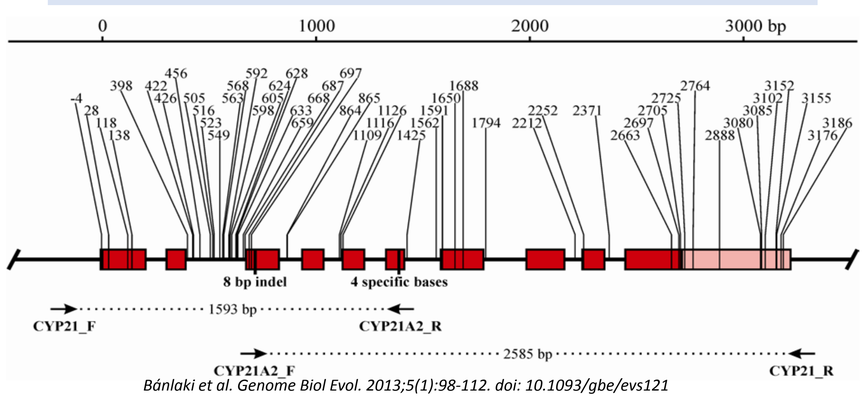
Background

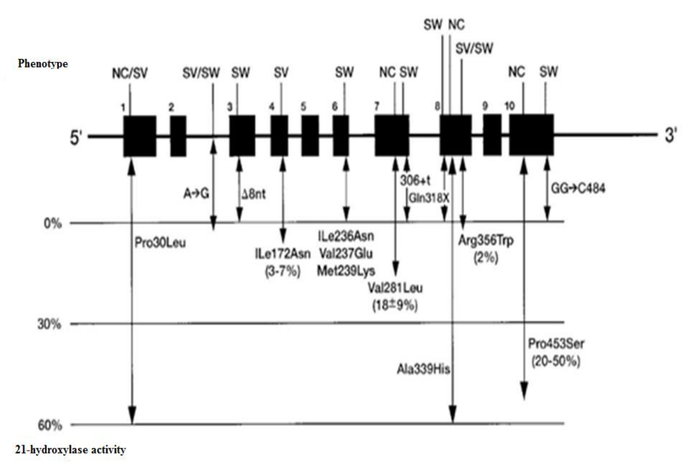
Congenital adrenal hyperplasia (CAH) is a rare (prevalence 1:15000), autosomal recessive disorder caused by 21-hydroxylase deficiency in 95% of all cases. This disorder is related to the mutation of *CYP21A2* gene that is located in a multiallelic complex called RCCX module showing tandem copy number 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.



Bánlaki et al. Genome Biol Evol. 2013;5(1):98-112. doi: 10.1093/gbe/evs121

Objective

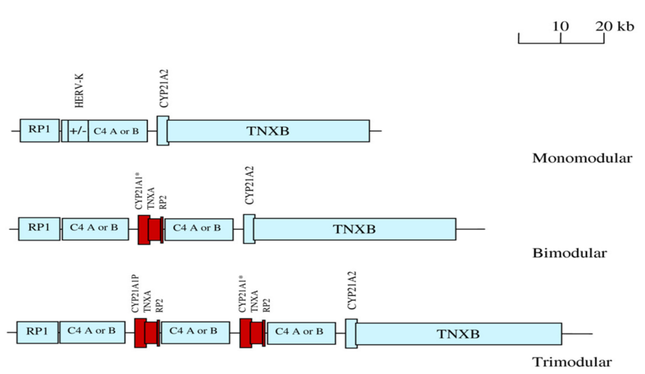
1. To analyze the most common mutations of the *CYP21A2* gene
2. To determine the copy number of the *CYP21A1P* and *A2* genes in our patients clinically diagnosed with CAH



Williams Textbook of Endocrinology

Patients and Methods

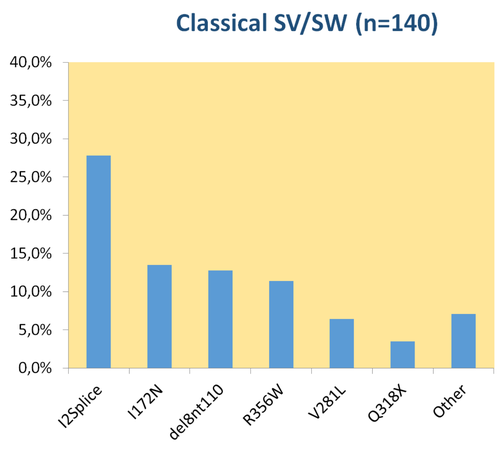
We studied 111 clinically diagnosed CAH patients (45 salt wasting, 25 simple virilising and 41 non classical/late onset). The most frequent mutations Δ8bpE3, P30L, IVS2-13A/C>G, I172N, R356W, Q318X) of the *CYP21A2* were screened by **allele-specific PCR**. The copy number of *CYP21A2* and its pseudogene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

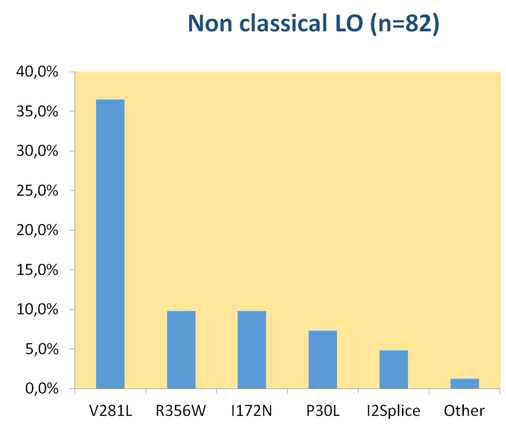


Sweeten et al. BMC Medical Genetics 2008 9:1 doi:10.1186/1471-2350-9-1

Results

1. The occurence of the most frequent mutations after allele-specific PCR



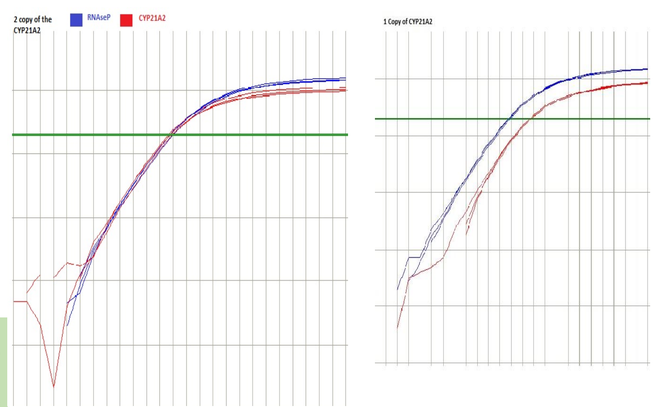


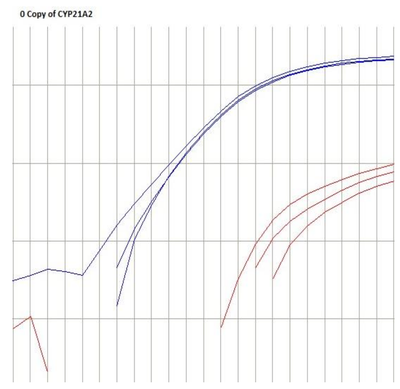
In the **classical form** among the examined 140 chromosomes we found **deletions in 39,3%,** the I2 splice in 27,8% and in 42,1% one of the most 5 frequent mutation was detected. By using complex molecular biological analysis 58 of 70 (**82,8%**) cases were resolved.

In the **non-classical cases deletions in 20,7%,** I2 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.

2. Copy number of the CYP21A2 gene

CN CYP21A2	Classical Phenotype (SW,SV n=70)	Non classical Phenotype (LO n=41)
0	**9 (12,9%)**	**0**
1	**37 (26,4%)**	**17 (20,7%)**
2	**24 (17,1%)**	**23 (28%)**
3	**0**	**1 (1,2%)**





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.

The authors acknowledge the financial support from Hungarian Research Found (OTKA PD100648) and from National Development Agency (KTIA_AIK-2-2012-0010)