Methodological considerations into the approach for genetic diagnostics of CAH in a girl with SW form and relatively higher needs of mineralocorticoids

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Introduction

The vast majority (80-95%) of congenital adrenal hyperplasia (CAH) cases are due to mutations in the *CYP21A2* gene encoding 21- hydroxylase(1). The residual activity of the gene defines the clinical form. Routine mutational screening of *CYP21A2* defects is shown to effectively support and complement the screening, biochemical and clinical diagnostics of newborns with CAH(2). According treatment consist of glucocorticoids and if necessary mineralocorticoids(3).

Methods

We evaluated a patient, identified by the 17-OHP (Delfia®) Neonatal Screening Program in Bulgaria. The screening diagnosis was confirmed clinically and biochemically. G-banding was performed to determine the gender. Additional genetic tests were conducted - MLPA (multiplex ligation-dependent probe amplification) and direct sequencing of the whole *CYP21A2*.

Objective

We aimed to characterise the phenotype of a girl with severe salt loosing CAH due to compound heterozygosity of four mutations on *CYP21A2.*

Results

Clinical report	CAH diagnostics
A girl born after first, pathological pregnancy with severe asphyxia and aspiration of amniotic fluid at birth; Apgar 1 min-3, intubated by the 10th min.	Neonatal 17OHP at day 7-675.7 nmol/l (ISNS < 20nmol/l)
BW 3340g, 38 G.A., BL 50 cm.	Na^+ (139-138), K^+ (4,7-5,6) Cl^+ (98-98) - day 1 and day 9
Ambiguous genitalia-Prader 4 (Fig.1)	Karyotype- 46XX (G-banding).
Multifocal infectious syndrome (Interstitial pneumonia, hepatosplenomegaly, tachycardia and pulmonary hypervolemia, urinary infection-treated successfully. Negative microbiological blood test-excluded TORCH. Gastroesophageal reflux.	Treatment starts at day 10 - Urbason 21,6 mg/m²/d 0,1mg Cortinef ® , saline infusions.

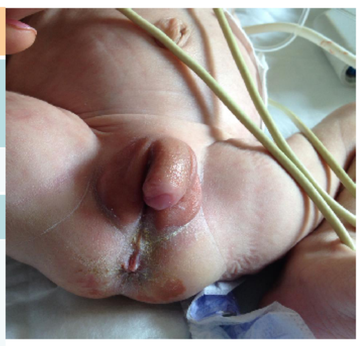

Fig. 1 Genital status

Age - weight-body area	17-OHP nmol/l	Na^+ mmol/l	K^+ mmol/l	Cl^- mmol/l	9 α-FC (mg)		Methylprednisolone (mg)		Hydrocortisone (mg/m²/d)	
						Adapted to		Adapted to		Adapted to
13d - 2680g-018m²		127	7,2	93	1x0,1	1x0,15	3x1	2x2		
45d - 2745g-0,19m²	2p.m-453;10p.m-125;6a.m.-174	134	6,5	91	1x0,1	1x0,1			30,9	25,7
3m - 4000g	70	124	8	90	1x0,1	1x0,15	2x2			3x2mg
1y- 6635g-0,34m²	1,7	145	3,8	104		1x0,1			17,6	14,6
1y 2m – 7050g-0,355m²	0.5	147	4,8	109	1x0,1	1x0,1			14,0	11,27

Genetic tests confirmed classical type of CAH due to 21-hydroxylase deficiency with compound heterozygosity (c.113G>A, Pro31Leu, Del8 E3-inherited from the mother; Q118X inherited from the father) (Fig.2). SW phenotype is determined by Del8 E3 (frameshift) and Q118X, both of them affecting the hem binding site and leading to <1% enzyme activity (Fig. 3, Fig. 4, Fig. 5)

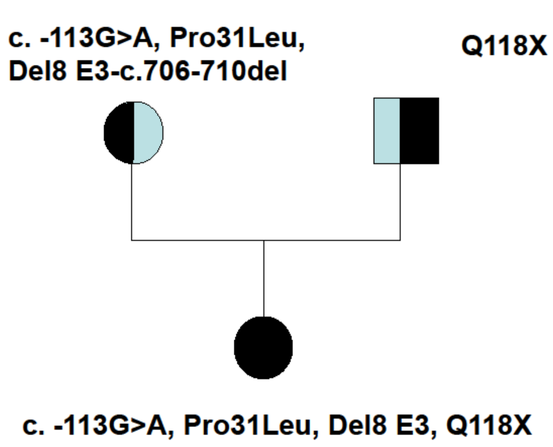

Fig. 2 CYP21A2 mutational analysis of the proband's family.

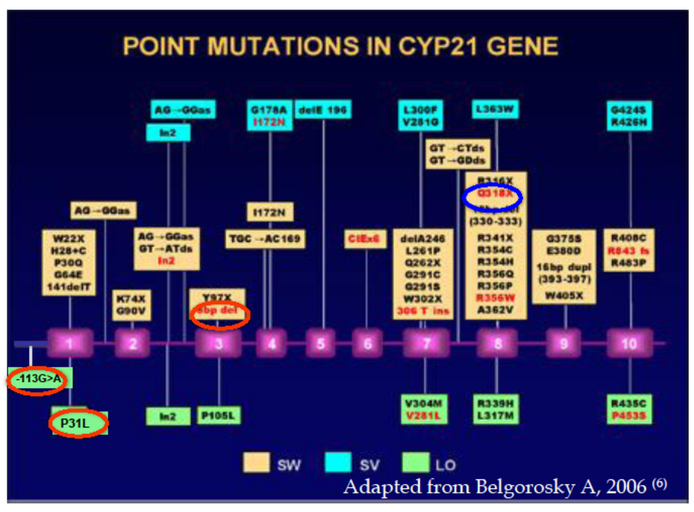

Fig. 3 Exon localization of CYP21A2 mutations in the patient.

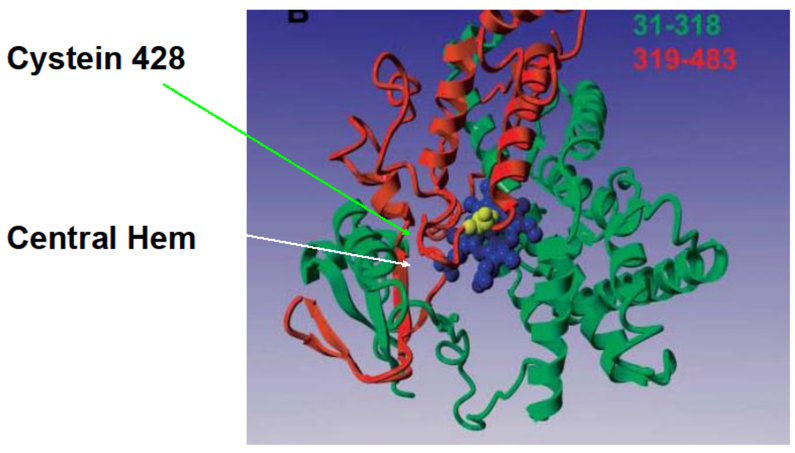

Fig. 4 Disruption of hem or substrate binding and weakening of structural stability by Q318X mutation causing enzyme activity less then 1%.

Janner et al., 2006 (7)

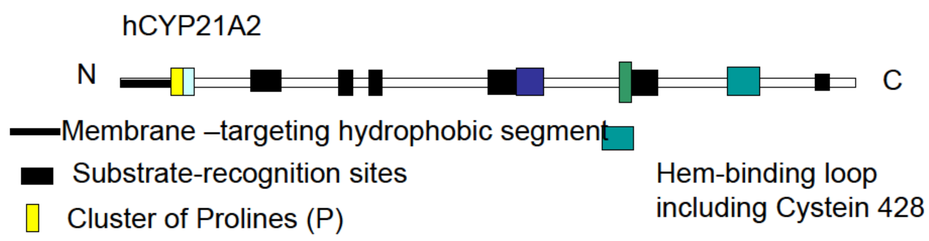

Fig. 5 P31L substitution located in the cluster of Prolines causes disruption of interactions, which are compensated by other residues, leading to enzyme activity more than 50%.

Haider et al., 2013 (8)

Conclusions

This case present an opportunity to investigate the benefits from using genetic screening for studying the severity of the clinical phenotype related to compound heterozygosity. Further methodological approaches for diagnostics of patients requiring higher doses of mineralocorticosteroids should be investigated.

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Grant 30/2013, Grant 42D/2013, Medical University Sofia <stoeva_i@abv.bg>