

Rapid Molecular Diagnosis of CAH by Strip Hybridization Assay in DEMPU

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INTRODUCTION AND OBJECTIVES

- ✓ Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder in which more than 90% of CAH cases are caused by mutations of the 21-hydroxylase (CYP21A2) gene and a nonfunctional pseudogene (CYP21A1P); CYP21A1P makes the genetic testing for the active gene difficult, as these two genes consist of 10 exons and show a high homology with a nucleotide identity of 98% in their exon and 96% in their intron sequences. Most of the inactivating mutations are generated by unequal crossing over or gene conversion between CYP21A2 gene and CYP21A1P. As a result, complete gene deletions/large gene conversions/ 8-bp/single point mutations are manifested with severe phenotypic anomalies in patients with CAH [1].
- ✓ Due to high consanguinity rate in the Egyptian community amounting to 35.3%, CAH is common in our population. Early and rapid diagnosis of CAH can be life-saving in cases of salt wasting (SW) and contributes to proper sex assignment in simple virilizing (SV) forms ; as salt wasting crises that are usually misdiagnosed in our communities as gastroenteritis, especially when the biochemical assay results are not conclusive.
- ✓ The aim of this study is to determine the mutational spectrum by strip hybridization assay in Egyptian CAH patients attending DEMPU.

METHODS

- Study group included 98 Egyptian CAH patients, diagnosed and registered since 1998 as follow up cases of DEMPU. Medical history was reviewed and a complete data sheet for each patient was created including presenting phenotype and the age of presentation, the initial and definitive sex designation and the karyotype. Hormonal results mainly elevated 17OH progesterone and other hormones were the basis of CAH diagnosis.
- Patients were classified into the SW, and SV by their phenotype, clinical history, and hormonal profile. circulatory shock and dehydration were considered clinical evidence of SW. For the late-onset (LO) cases the diagnosis was made after the first year of life, including some cases in which neonatal virilization was present but not observed initially. Symptoms and signs of androgen excess are presented as the age of onset and degree of growth acceleration, the advancement of bone age, and age of onset of precocious penile growth/clitoral enlargement or pubic hair growth.
- Genomic DNA was extracted from peripheral blood samples on EDTA (QiaAmp DNA extraction kit, Qiagen). Analysis of 11 common mutations in all patients was carried out using reversed hybridization assay strips from VIENNA LAB GmbH, Vienna, Austria according to manufacturer's instructions [2].

RESULTS

- 98 CAH patients tested by strip hybridization assay, 15 of them were confirmed in Vienna labs (Vienna, Austria), along with 15 controls. Collectively, patients were 70 females and 28 males, male: female ratio;1:2.5, with ages 6.2 5.3 and 4.3 4.9 years, respectively. Out of the 98 cases; 77 (78.5%) presented with SW, SV 19 (19.3%) and 2 (2%) cases of LO.
- 10 point mutations were encountered in the CYP21A2 gene accounting for 88.7 % (87/98) of the screened patients, 11 (11.2%) patients show complete failure in the strip assay for differential diagnosis as complete deletion of the gene or amplification failure [2]. Results of the CYP21A2 gene genotyping for the 87 cases with successful strip assay are shown in table (2). The R483P point mutation was not encountered in any of the studied cases. Prader stage was considered as an indication of severity of disease [3] and thus was plotted against the 5 most common genotypes in fig (1). Analysis of the most frequently encountered genotypes in relation to their presenting clinical phenotype is seen in figure (2).
- The most common mutations encountered were homozygous mutations for I2 splice, this mutation alone accounted for 52.8%(46/87) of the analyzed cases. The most common combination seen is the combined homozygosity of three mutations namely the p.P30L, I2 splice and the 8bp deletion which accounted for 12.6%(11/87) of the cases. The third most frequent genotype interestingly, was the "normal" genotype, who are negative to all listed mutations, accounting for 11.5%(10/87) of cases; meanwhile these cases expressed the typical phenotype of the disease, 5% of the cases showed a single heterozygous mutation; not sufficient to explain the observed phenotypes either. Preliminary data suggest a probable ethnic specific mutation exists, that will be further studied by the research team to record the Egyptian peculiar mutations.

CONCLUSIONS

- ✓ Strip hybridization assay method commercially available will help easy, rapid and cost effective testing for CAH patients. This might be the first step to facilitate its establishment as a routine molecular diagnostic method and providing genetic counseling for families of CAH patients.
- ✓ However, these data are still premature to answer the most important question still lagging in literature, which is to determine which genetic defect would be likely to present with a detrimental effect. Answering this question will help guide premarital and prenatal counseling.

Table 1: Demographic and clinical data of studied CAH patients (n=98).

	SW (n=77)		SV(n=19)		LO (n=2)		
Presentation age days (mean SD)	59.17	258.2	11.05	28.65	2190.0	0.0	
Current age (median (2.5 th -97.5 th centiles)	3.8 (0.1-18.9)		5.7(1.0-22.1)				
Sex	M	F	M	F	M	F	
	Rearing	56	21	8	11	0	2
	Karyotype	25	52	2	17	0	2
Definitive	25	52	3	16	0	2	
(+)Consanguinity n (%)	53 (68.8%)		11 (57.8%)		2 (100%)		
Similar family condition n(%)	32 (41.5%)		8 (42.1%)		1 (50%)		
Atypical genitalia	50(64.9%)		17 (89.4%)		1 (50%)		
Precocious puberty	5 (6.5%)		3 (15.7%)		1 (50%)		
Hirsutism	3 (3.8%)		1 (5.26%)		2 (100%)		
Na mmol/l (mean SD)	120.8 8.3		136.9 3.6		140.5 2.1		
K mmol/l (mean SD)	6.4 1.0		4.6 0.7		4.2 0.14		
17 OH progesterone	17.6 10.2		22.69 17.1		14.5 6.4		
Corrective surgery	42 (54.5%)		15 (78.9%)		1 (50%)		
Prader stage	I	4 (5.1%)		2 (10.5%)		1 (50%)	
	II	1 (1.2%)		2 (10.5%)		0	
	III	39 (50.6%)		11 (57.8%)		0	
	IV	7 (9.1%)		1 (5.2%)		0	
	V	0		1 (5.2%)		0	

M: male, F: female, +consanguinity: positive consanguinity, SW: salt wasting, SV: simple virilizing, LO: late onset

Table 2: The different genotypes seen in studied cases (total number= 87)

	P30L	I2 Splice	8BP del	I172N	V281L	L307 frameshift	Q318X	R356W	P453S	cluster E6
Normal	68	41	71	72	71	81	67	83	86	85
Hetero	5	15	4	7	8	2	9	2	1	2
Homo	14	31	12	8	8	4	9	2	0	0

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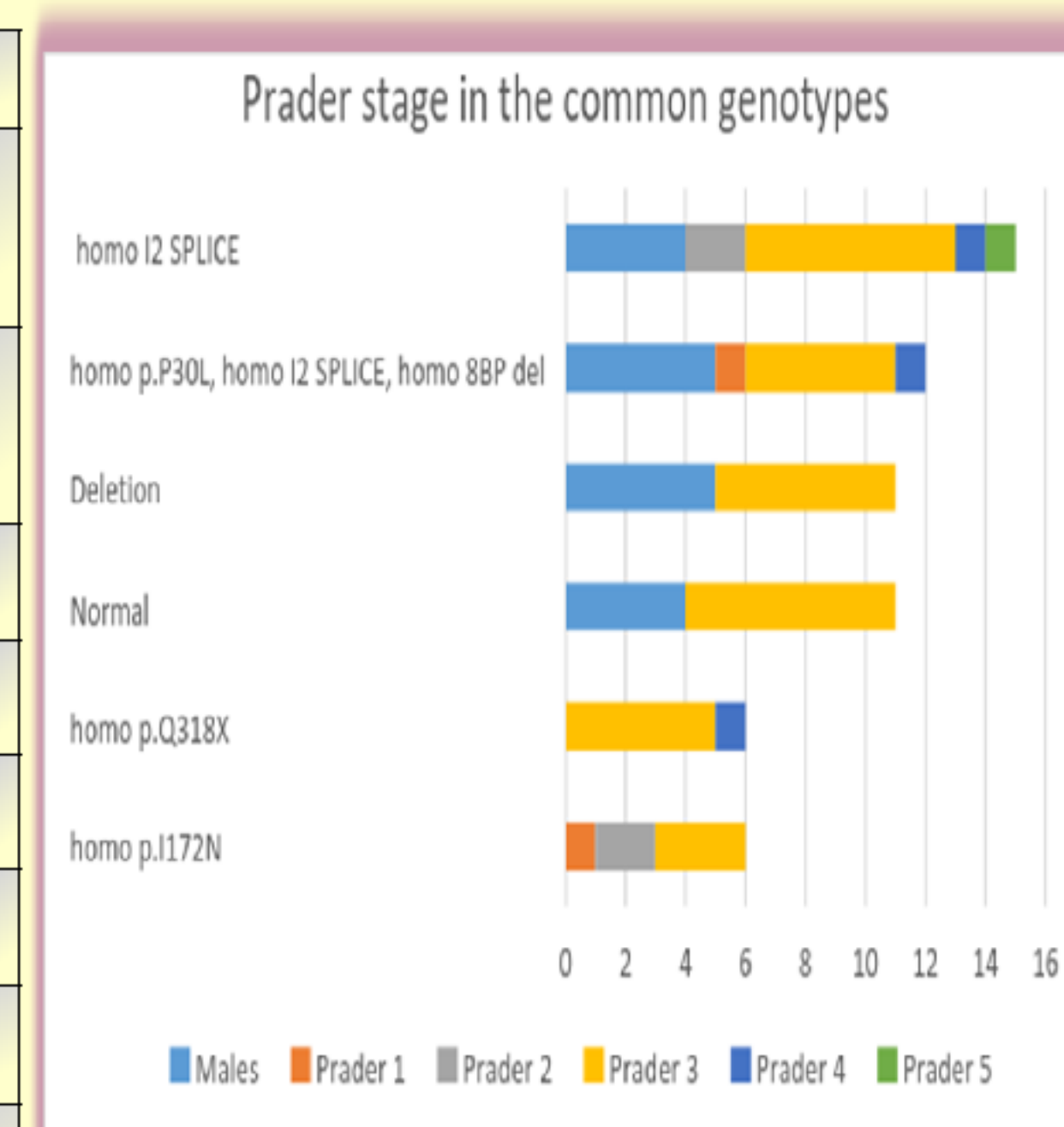


Fig (1): Prader staging in the most common genotypes.

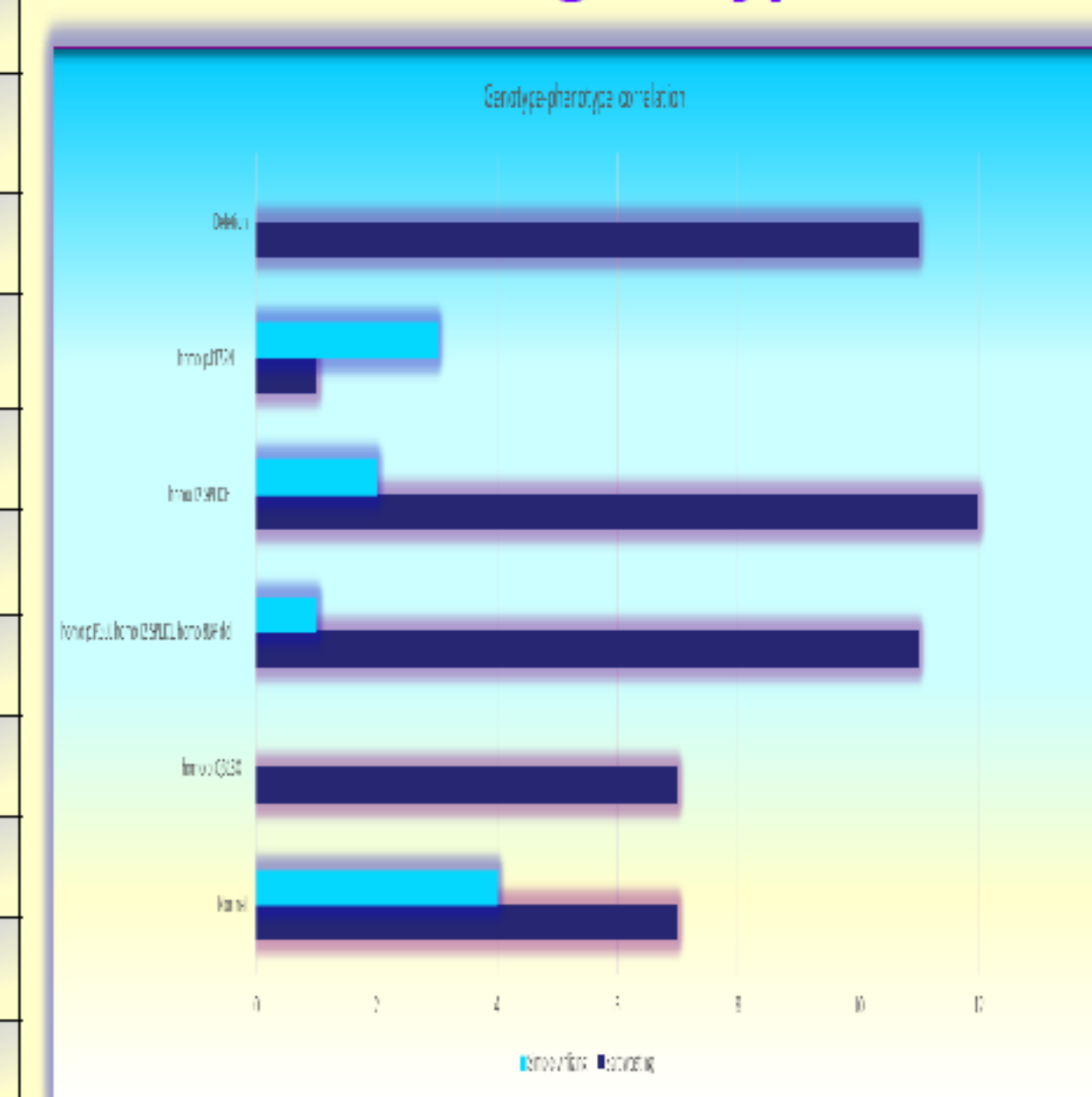


Fig (2): Genotype-phenotype correlation in selected common genotypes

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