P1-P38
Session name: Adrenal
Abstract number: 624

Twenty Years Experience in Congenital Adrenal Hyperplasia: Clinical, Hormonal and Molecular Characteristics in a Large Cohort

Mirela Costa de Miranda1, Daniel Fiordelisio de Carvalho, MD1, Larissa Garcia Gomes, MD, PhD1, Guiomar Madureira, MD, PhD1, Jose Antonio Marcondes, MD, PhD1, Ana Elisa C Billerbeck1, Andresa De Santi Rodrigues1, Paula F Presti2, Hilton Kuperman2, Durval Damiani, MD, PHD2, Berenice B Mendonca1 and Tania A Bachega, Professor1

[1]Laboratório de Hormônios e Genética Molecular LIM/42, Unidade de Adrenal, Disc. de Endocrinologia e Metabologia, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, (2)Unidade de Endocrinologia Pediátrica do Instituto da Criança do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

The authors declare no conflicts of interest

Introduction and Objective

Most congenital adrenal hyperplasia (CAH) patients carry mutations derived from conversion events involving the pseudogene, and the remaining carry new mutations varying according to ethnicity. In CAH is observed a good genotypephenotype correlation, allowing the use of molecular analysis in clinical practice.

Objective: to review the molecular diagnosis in a large cohort of CAH patients in order to create a diagnostic panel in our population.

Design and Methods

DNA samples were extracted from 480 patients (158 SW, 116 SV, 206 NC); 14 point mutations were screened by allelespecific PCR and large gene rearrangements by Southern blotting/MLPA; *CYP21A2* sequencing was performed in those with incomplete genotype. Gene founder effect was analyzed through microsatellite studies. Patients were divided into 4 genotypes, according to *in vitro* enzymatic activity (Null, A:<2%, B:37%, C:>20%).

Results

Targeted methodologies identified mutations in both alleles in 89% of SW, 86% of SV and 80% of NC patients. *CYP21A2* sequencing allowed genotype definition in 100% of classical and 87% of NC patients. Nine percent of alleles carried large gene rearrangements and 87% point mutations. The most frequent mutations in SW, SV and NC forms were I2 splice (21%), p.I172N (7.5%) and p.V281L (27% of alleles), respectively. Seven rare mutations (p.G424S, p.R408C, IVS22A>G, p.Ser170fs, p.R426H, p.H365Y, p.W19X) and a novel variant (p.E351V) were identified in 11% of alleles. Gene founder effect was observed in all but the p.W19X. Genotypes Null, A (I2 splice), B and C comprised mainly patients with SW (88%), SW (70%), SV (98%) and NC form (100%), respectively; 31 NC patients remained with incomplete genotype. Among NC patients, median basal 17OHP levels varied from normal to those that overlap with the classical form. The median basal 17OHP level was significantly higher in genotype A/C [median 17.5 ng/mL] than in C/C [median 7.6 ng/mL] as was ACTHstimulated 17OHP level [P=0.005].

The lowest stimulated17OHP level in group C was 11 ng/mL and the best cutoff to identify NC patients carrying compound heterozygosis for severe mutations was 44.3ng/mL (Area Under Roc curve= 0.701 [0.5970.804]) [P=0.001].

Genotype-phenotype correlation

	SW	SV	NC	genotype-phenotype correlation
Null	60	5	0	95.3%
A	111	26	0	70%
B	0	64	1	98%
C	0	0	178	100%

Conclusion

We identified a good genotypephenotype correlation providing useful results regarding prediction of disease severity and genetic counseling; moreover, we suggest that 17OHP levels could predict carrier status for severe mutations. Sequencing is essential to optimize molecular diagnosis in our population, considering the high frequency of gene founder effect mutations.

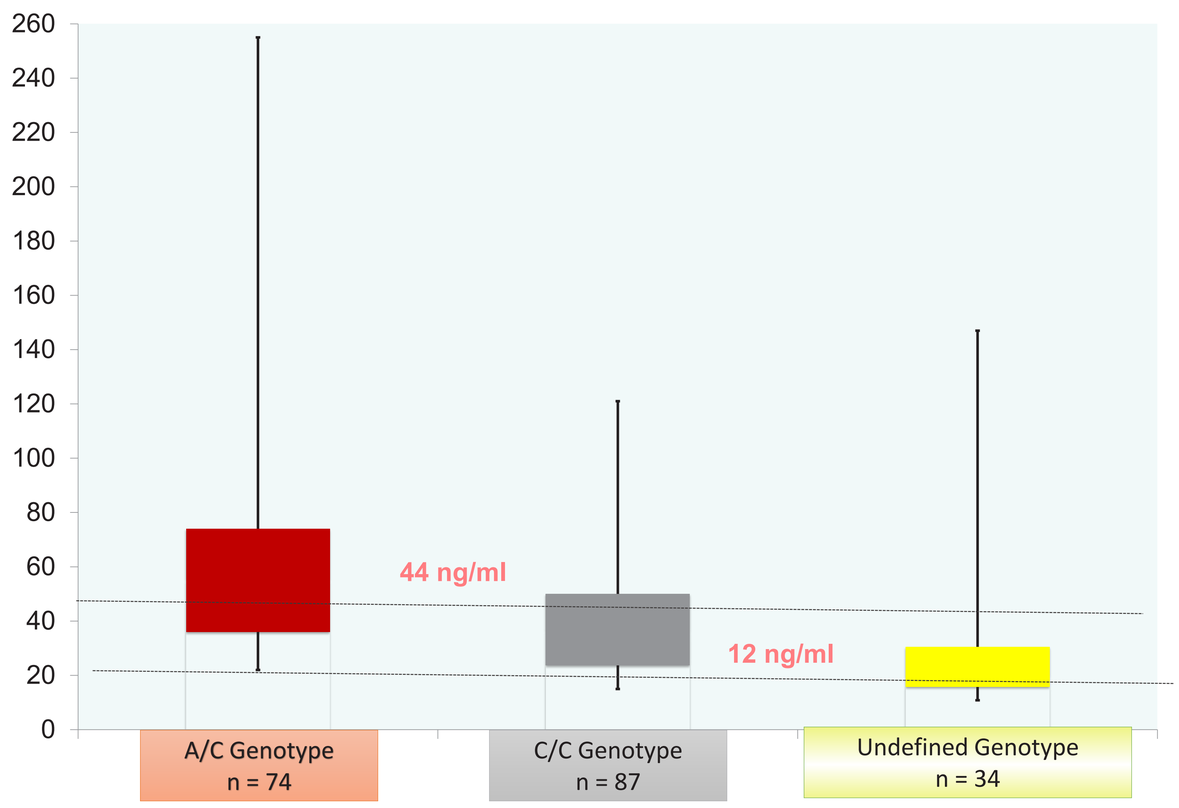
Frequency of CYP21A2 mutations in Brazilian CAH patients and in other countries

Country or Region	Brazil	Argentina	Portugal	Chile	Germany	USA	Mexico	Mid. Europe
	This study	*Marino*	*Friães*	*Fardella*	*Krone*	*Finkielstain*	*Ordoñez*	*Dolzan*
Mutations / Nº alleles	856	866	112	164	310	426	94	864
CYP21A2 Del or Conv	9.0	11.2	25.9	19.5	27.4	31.9	1.0	30.6
p.P30L	0.6	0.7	1.8		2.6	0.8	8.5	3.7
IVS2-13A/C>G	21.1	20.6	9.8	15.8	30.3	23.4	47.9	31.2
p.G110EfsX21 (E3Δ8bp)	1.8	0.8	2.7		1.6	0.5	2.1	1.0
p.I172N	7.5	8.2	9.8	7.3	19.7	12.6	11.7	14.5
p.I235N, p.V236E, p.M238K (E6 cluster)	1.2	2.0		1.8	1.0	1.1	0.0	0.3
p.V281L	26.6	26.2	25.9	2.4	2.9	12.6	8.5	3.4
p.Leu307fs (InsT)	2.2	*	4.4	0.0	0.3	0.3	1.1	1.6
p.Q318X	6.1	6.7	6.3	7.9	4.8	3.3	4.2	2.6
p.R356W	5.4	4.2	1.8	9.7	4.5	3.6	7.4	2.4
p.P453S	1.4	1.4			0.3	0.5	2.1	0.7

***CYP21A2* mutations not derived from pseudogene events: Novel mutations and mutations with gene founder effect**

					Microsatellites[a]	
ENSP00000408860	*ENST00000418967*	*Frequency (%)*	*Patients+ (n)*	*Founder effect*	*D6S273*	*TAP-1*
p.G424S	c.1273G>A	0.6	6	+	1	5
p.Ser170fs	c.511_512insA	0.5	4	+	3	6
p.R408C	c.1225C>T	0.5	4	+	5	5
IVS2-2A>G		1.2	10	+	3	1
p.H365Y	c.1096C>T	0.2	2	+	2	5
p.R426H	c.1280G>A	0.8	7	+	6	3
p.W19X	c.59G>A	0.2	2	-	-	-
p.E351V	c.1055A>T	0.3	3	+	4	5

ACTH stimulated 17OHP levels in NC patients according to to genotype



DOI: 10.3252/pso.eu.55ESPE.2016