SOCIEDAD LATINOAMERICANA DE ENDOCRINOLOGIA PEDIATRICA

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1

ANALYSIS OF POINT MUTATIONS (PM) OF CYP 21B GENE IN PATIENTS WITH 21-HYDROXYLASE DEFICIENCY (CAH). A. Dardis; N. Saracco; M.A. Rivarola; A. Belgorosky. Laboratorio de Investigaciòn, Hospital de Pediatria Garrahan, Buenos Aires, Argentina.

It has been described that only 25% of lesions in the active CYP 21B gene can be detected by Southern blotting, i.e., 30 KPB deletions and large gene conversions (D-C). The remaining 75% are probably PM. The 8 most frequent PM are present in the pseudogene, suggesting that they are the consequence of gene microconversions. D-C, as well as several PM or microconversions (codon 318, C $\longrightarrow$ T; codon 234-238, T-T-T $\longrightarrow$ A-A-A; exon 3, -8BP and codon 172, T $\rightarrow$ A, but in only 4 patients (p) were studied in 20 p with CAH (12 salt losers, SL, 2 simple virilizing form, SV, and 2 late onset non-classical, NC, CAH). By POR technique, D-C were found in 3 p (6 alleles, a) while the alteration in codon 234-238 was detected in 1 p (2 a). PM in codon 318 was detected in 4 p (4 a) after digestion with restriction endonuclease (RE) PST 1 followed by fragment analysis, and in exon 3 in 2 p (2 a) with RE ALU-1. Codon 172 PM was discarded by the SSCP technique in the 4 p studied. Alterations were detected in 50% of SL, 50% if them being D-C, 33% codon 318 PM and 17% exon 3-8BP deletions. One p with SV was homozygous for codon 234-238 M. It is concluded that by combining PCR and RE fragment analysis several of the most frequent PM can be studied in a simple and rapid way when a change in a normal restriction site has taken place.

2

CLINICAL AND GENETICAL CHARACTERISTICS IN CLASSIC CONGENITAL ADRENAL HYPERPLASIA DUE TO. 21-HYDROXYLASE DEFICIENCY. PRELIMINARY DATA IN 12 BRAZILIAN FAMILIES. G. Guerra(*); M.P. Mello(**); S.B. Farah (**) M. Araujo(**); M.T.M. Baptista(*); S.H.V.L. Marini (*). Disciplina de Endrinologia Pediàtrica (*) e Centro de Biologia Molecular e Engenharia Genètica (**) - UNICAMP - Campinas - SP - BRASIL.

The genetic organization of the 21-OHase (P450c21) complex in CAH is well characterized, but the literature studies vary depending on the population. This study was carried out to determine the genic alterations of the P450c21 complex in brazilian families. During 6 months, 12 families were analyzed with 15 affected individuals with classic 21-OHase deficiency. Blood samples of all family members were tested for hibridization on conventional Southern blot with TaqI digests of DNA with gene probes for P450c21 and C4. Among the 15 affected individuals (9F:6M, with positive family history and consanguinity in 5/12), 8 had the simple virilizing and 7 had the salt-wasting form. The most frequent genic alteration was the point mutation of the 21B gene with normal 21A gene (50%), a result similar to those of the literature. The absence of deletion of the 21B gene and the finding of 30% of convertion in 21B gene indicate the necessity of new studies with more patients.

3

ADRENAL IMAGE STUDIES IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA DUE TO 21-HYDROXYLASE DEFICIENCY. TAS Bachega; J. Mattieli; G. Madureira; CA. Suslik; GC. Gomes; E. Secaf; W. Bloise; IJP. Arnhold; BB. Mendonca. Department of Endocrinolgy and Radiology, Hosp. Clinicas FMUSP, Sao Paulo, Brazil.

The occurrence of nodules in patients with adrenal enzymatic defects is reported in the literature, but its frequency and etiology are not clear. We studied 14 patients (10F,4M) with congenital adrenal hyperplasia due to 21-hydroxylase deficiency with mean age 13,3y (2,4-36y). Two patients had the salt-losing form, 7 simple virilizing and 5 symptomatic non classic form. Image studies were carried out before treatment in 2 patients and after 1,4 to 14 y of therapy in 12 (mean 5,7y). Two had computed tomography and the other 12 magnetic resonance imaging. Treatment was classified according to 4 quarterly testosterone and adrostenedione measurements during the year before imaging in: (E) Excellent -all levels normal (1 case); (G) Good -one high value (4); (R) Regular -two high values (6); (P) Poor-three or more abnormal values(1). Both patients evaluated before therapy had enlarged adrenals one with 0.9 cm nodule that disappeared after one year of therapy; of the 12 patients studied during therapy 6 (50%) had an enlarged left adrenal and one patient with P control both adrenal enlarged and a 0,9 cm nodule in the L. adrenal. The patient with E control had normal adrenals and the patients with G and R controls had normal or enlarged adrenals. We conclude that adrenal enlargement and nodules are related to control suggesting an effect mediated by ACTH.

4

MUTATIONS OF THE ANDROGEN RECEPTOR GENE IN THE ANDROGEN INSENSITIVITY SYNDROME. (AIS) IJP. Arnhold, BB Mendonca, ACM. Rigon, W. Bloise, TM. Claude,J. Unidade de Gonadas e Intersexo, Disc. de Endocrino. Hosp. das Clinicas,Sao Paulo,Brasil e School of Hygiene and Public Health,Johns Hopkins University,Baltimore,Maryland, USA.

Androgen receptor (AR) gene was studied in 8 patients with AIS diagnosed clinically and biochemically.All had 46,XY karyotype and bilateral testes.Four with complete form had normal female external genitalia and breast development at puberty. Four with partial AIS had ambiguous genitalia and gynecomastia. In order to identify the AR mutations individual exons of the human AR gene were analyzed by denaturing gradient gel electrophoresis after amplification of genomic DNA by PCR. Altered exons were sequenced.

Case	CA (Y)	Type	T* ng/dl	MOLECULAR DEFECTS Exon	Mutation	Aminoacids
1	14	Cais	790	6	R778W	CTG-- TGC
2	17	Cais	317	6	M807V	ATG-- GTG
3**	28	Cais	286	7	R855C	GCG-- TGC
4**	16	Cais	1033	7	R855C	GCT-- TGC
5	25	Pais	1100	5	Y763C	TAG-- TGC
6	0.1	Pais	332		Absent	--
7	9	Pais	--		Absent	--
8	19	Pais	--		Absent	--

* Basal (aduls), after hCG (prepubertal patients)
** Belong to the same family

In 5 of the 8 patients a point mutation in the hormone binding domain of the AR gene was found.With this methodology new mutations of the AR gene were detected in most of the patients studied.