ABSTRACT CATEGORIES

● Oral Presentations
* Poster Symposia Presentations

Steroids, Adrenals, Gonads

● 56

MUTATIONAL SPECTRUM OF THE STEROID 21-HYDROXYLASE GENE.
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Lesions in the gene encoding steroid 21-hydroxylase (CYP21) result in defective adrenal steroid synthesis; the severe forms are known as congenital adrenal hyperplasia. To facilitate complete characterization of mutations in this region of tandemly repeated genes, we have developed selective PCR amplification and direct sequencing of full-length steroid 21-hydroxylase genes. This technique identifies known mutations, characterizes or excludes unknown mutations, and gives an estimate of gene copy number. Genetic defects in the 21-hydroxylase genes in a patient material representing 182 unrelated chromosomes have been studied. For 138 of these, HLA class II typing was performed, and for 86 chromosomes the gross structure of the C4 / 21-hydroxylase locus was determined. Thus, the location of the different mutations on different haplotypes are described. Functional consequences of individual alleles and combinations of alleles could be determined *in vivo* by studying individuals with known gene copy number, including hemizygous individuals. Genotypes showed good correlation to the clinical course of the disease. Six additional defective alleles were found, and several polymorphisms were shown to be neutral. The six mutations found are not present in the pseudogenes hitherto reported. Sequencing of pseudogenes (CYP21P) showed that this gene displays an equal degree of polymorphism to that of CYP21, and that two of the six above-mentioned mutations were present at low frequency. This implies that also the rare mutations can spread via CYP21P and can be expected to arise independently in unrelated individuals.

57

NORMAL PUBERTY IN X-LINKED CYTOMEGALIC CONGENITAL ADRENAL HYPOPLASIA (CCAH). T.M. Strom, W. Rabl, R. Schwertner, R. Senekowitsch[1], K.E. Davies[2]. Kinderklinik and [1]Nuklearmedizinische Klinik, Technische Universität, München, Germany, and [2]John Radcliffe Hospital, Oxford, UK.

X-linked cytomegalic congenital adrenal hypoplasia (CCAH) is regularly associated with hypogonadotropic hypogonadism.[1,2] To our knowledge, we are reporting for the first time a boy with well documented X-linked CCAH and normal pubertal development. The patient's brother had died at 5 $^{6}/_{12}$ years of age from undiagnosed adrenal insufficiency of recent onset due to histologically confirmed CCAH. Our patient developed primary adrenal insufficiency at 6 $^{6}/_{12}$ years of age, and has been doing well ever since on physiological replacement doses of hydrocortisone and fludrocortisone. At the time of diagnosis, there were negative or normal results for adrenal and other endocrine autoantibodies, very-long-chain fatty acids, serum and urinary glycerol, serum triglycerides, CK, and urinary organic acids. At 15 $^{6}/_{12}$ years of age, audiometry was also documented to be normal.[3] Spontaneous onset of puberty was evidenced by testicular enlargement and appearance of pubic hair in his 11th and 14th years of life, respectively, and by a corresponding growth spurt. At 15 $^{8}/_{12}$ years of age, he had an adult sized phallus, testicular volume of 20 ml, and pubic hair stage IV (Tanner). Serum FSH (4.8/7.2 U/l) and LH (4.9/20 U/l) before and 30' after LHRH (60 µg/m^2 i.v.), a LH night profile (3 peaks up to 7.6 U/l), and serum testosterone (493 ng/dl) were within the normal range for adult males. Although molecular genetic studies have so far failed to identify a deletion on the short arm of the X chromosome in our patient, this unique case of normal puberty in CCAH supports the suggestion[2,4] that a separate gene locus for hypogonadotropic hypogonadism is located distal to the glycerol kinase and CCAH genes.

1) Prader A et al.: J Pediatr: 1975;86:421-422. 2) Matsumoto T et al.: Am J Med Genet: 1988;31:603-616. 3) Zachmann M et al.: Eur J Pediatr: 1992;151:167-169. 4) Gonnewardena P et al.: Clin Genet: 1989;35:5-12.

58

IDENTIFICATION OF A NEW MUTATION IN STEROID 11β-HYDROXYLASE DEFICIENCY.
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Steroid 11β-hydroxylase (P450$_{11\beta}$) deficiency, an autosomal recessive hereditary disease, accounts for about 8% of congenital adrenal hyperplasia. Recently, *CYP11B1*, the gene for steroid 11β-hydroxylase (P450$_{11\beta}$) has been isolated and its nucleotide sequence determined. In the present study, we have carried out a molecular genetic study on a Japanese patient who is an offspring of a consanguineous marriage. We amplified 9 exons of *CYP11B1* from the genomic DNA of the patient by polymerase chain reaction (PCR). Nucleotide sequence analysis of the PCR products revealed occurrence of a point mutation in exon 2 which leads to the formation of a premature stop codon. Furthermore, we performed genomic Southern blotting analysis and restriction fragment length polymorphism analysis of the PCR products amplified from exon 2 in *CYP11B1* of his family members. The results indicated that the patient is homozygous and his unaffected parents are heterozygous as for the mutation. White and his coworkers are the first to find out a missense point mutation near the heme-binding locus of *CYP11B1* (White et al. *J.Clin.Invest.* 1991.87:1664-1667), but our present findings provide the first molecular basis of this disorder caused by nonsense mutation in *CYP11B1*.

● 59

A NEW POINT MUTATION OF SRY GENE IN TWO SISTERS WITH 46 XY GONADAL DYSGENESIS
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The sex determining region of Y(SRY) is required for the male sex determination. Recently several mutations of SRY gene have been identified in 46XY gonadal dysgenesis(GD). All mutations reported so far are located within the putative DNA binding motif known as HMG box domain. We investigated SRY gene of four sporadic cases and two sisters in one family with 46XY GD by polymerase chain reaction and single strand conformation polymorphism and subsequent DNA direct sequencing. Four sporadic cases did not show any mutations in SRY gene, while two sisters in one family shared the same one base mutation T to A, which exists out of the putative DNA binding motif region of SRY gene. By this mutation, a codon TTG(Leucine) changes a stop codon TAG. Generation of this stop codon would be expected to make a truncated nonfunctional SRY gene products, and affect DNA binding activity ,resulting in 46,XY GD.

From these results it is concluded that in addition to the mutations in HMG box domain of SRY gene new mutation reported here cause 46 XY GD. Furthermore, this new mutation in this family will shed light on disclosing the mechanism of genetic transmission in the familiar cases of 46 XY GD.

* 60

CLINICAL, BIOCHEMICAL AND GENETIC STUDIES IN ANDROGEN INSENSITIVITY SYNDROME: A TWO YEAR STUDY. M.N.Patterson, H.R.Davies, J.A.Batch, D.M.Williams, B.D.Brown, I.A.Hughes. Paediatrics Department, Cambridge University, Addenbrooke's Hospital, Cambridge, UK.

The androgen insensitivity syndrome (AIS) is subdivided clinically into complete (CAIS) and partial (PAIS) forms. In the past two years, we have received 36 samples from confirmed cases of AIS: 16 CAIS, 20 PAIS. Patients with CAIS had normal female external genitalia, while those with PAIS typically had severe hypospadias, micropenis and a bifid scrotum. Eight of the CAIS cases had a family history of AIS. Androgen binding analysis, possible in 9 of the CAIS cases, revealed negative binding in 6 cases, thermolability and reduced binding affinity in 2 cases, and reduced binding levels in 1 case. Six of the CAIS cases were screened for androgen receptor gene mutation by single strand conformation polymorphism and 4 mutations found, all affecting the ligand binding domain. Carrier detection was possible based on the specific mutation or the polymorphic exon 1 repeat in the androgen receptor gene. In one case however, evidence for mosaicism at the polymorphic repeat was found in a female at risk of being a carrier. Diagnosis of PAIS depended on a normal age-appropriate response to hCG and where available, histologically normal testes. None of the PAIS cases had a positive family history. Androgen binding was studied in 10 cases: in 6 the results were normal (Kd: 1.08±0.25x10^{-10}M; Bmax: 1343±419x10^{-18} moles/µg DNA; thermolability: 16.2±7.5% reduction in binding at 40°C), in 2 cases Kd and thermolability were increased, and in 2 further cases thermolability alone was increased. Reduced levels of binding (Bmax, 366x10^{-18} moles/µg DNA) were found in a patient with simple hypospadias alone. One PAIS sample with elevated Kd (5.4x10^{-10}M), and thermolability (49%) was screened for mutation and a point mutation in exon E of the androgen receptor gene was identified. The range of phenotypes in PAIS, and the normal androgen binding in most samples, makes it likely that many cases of PAIS diagnosed by current criteria are not caused by androgen receptor defects. Genetic counselling can therefore only be offered in PAIS when a mutation has been identified as the cause of the condition.

61

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ASSESSMENT OF CORTISOL SECRETION IN CHILDREN WITH ASTHMA TREATED WITH INHALED STEROIDS.

Conventional assessment of adrenal function is achieved by measurement of 0800h serum cortisol, 24h urinary cortisol excretion and standard dose (250µg) ACTH(1-24) or insulin tolerance testing. Adrenal function was assessed as part of a growth study in 56 pre-pubertal (age range 4-12 years) children with asthma who were grouped according to treatment. The groups were: 1=non-steroid, n=13; 2=budesonide, n=20; 3=beclomethasone, n=20; prednisolone, n=3. The mean(SD) dose of inhaled steroid in groups 2 and 3 was similar: 762(525) and 560(281)µg/m^2/day respectively, p=0.15. 31 short normally growing children (Gp5) were used for comparison. Estimates of cortisol secretion were: 24h serum cortisol profile with sampling every 20 min to give mean, peak and 0800h values and in circadian rhythm by analysis of timing of early morning cortisol rise. A 24h urine was collected for total cortisol metabolites. The cortisol response to a low (500ng/1.73m^2) and standard (250µg/1.73m^2) dose of ACTH(1-24) was assessed by comparison of the rise in and concentration of cortisol at 20 min (low dose) and 60 min (standard dose). **Results:** There was no group difference in 0800h cortisol but mean 24h cortisol was lower in Gp2,3,4 (p=0.0005). Mean 24h cortisol correlated best with cortisol rise and concentration at 20 min after low dose ACTH (r=0.45, p=0.002; r=0.55, p<0.0001), time of early morning rise (r=-0.64, p<0.0001) and 0800h cortisol (r=0.36, p=0.002) but weakly with urinary cortisol excretion (r=0.29, p=0.08) and cortisol rise and concentration at 60 min after standard dose ACTH (r=0.28, p=0.3). We conclude that low dose ACTH testing gave the most accurate guide to physiological cortisol secretion as assessed by a 24h profile.