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XX MALES AND AMBIGUOUS GENITALIA : A LINK BETWEEN SRY AND ANDROGEN RECEPTOR (AR) GENE ?

Introduction. Most XX males, SRY positive, have a normal male phenotype. I some cases, XX males, SRY negative, are associated with ambiguous genitalia (AG). The aim of this study was to find out whether, in these patients, AR amount is diminished in target cells and whether partial androgen resistance is relevant to molecular abnormalities of the AR gene. **Patients and Method.** Three XX males with AG were studied. PCR of the SRY gene, known to be equated to the testis determining gene, was negative. AR binding capacity (Bmax) and dissociation constant (Kd) of the A-AR complex were determined on genital skin fibroblasts by Scatchard analysis. PCR coupled with SSCP analysis was performed on all exons (4-8) encoding for the androgen-binding domain (ABD) of AR. **Results.** Receptor-binding capacity was respectively 243, 206 and 162 fmol/mg DNA (N = 650 ± 200 fmol/mg DNA). Kd was respectively 1, 0.66 and 0.4 nM (N = 0.6 ± 0.3 nM). No band shifts were detected on the 5 exons studied. Known mutated exons of AR detected by SSCP were used as control. **Discussion.** In patients with partial androgen insensitivity syndrome and diminished receptor binding capacity, a mutation has been reported within the ABD. In the 3 XX males, AG seems to be due to a low level of AR, without any detected mutation in the ABD. In these XX males, SRY negative, AG is relevant to a low level of expression of the AR gene. We suggest that the product of the SRY gene could thus be a transcriptional factor of the AR gene. Production of AR by AR-negative COS cells transfected by SRY gene is now under investigation.

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RACIAL DIFFERENCE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS OF P450c11 GENE.Y.Nakagawa,H.Takeuchi,A.Kubota,H.Ogawa,N.Yamada* and Y.Igarashi,Department of Pediatrics,Hamamatsu University School of Medicine,3600 Handa-cho,Hamamatsu431-31,Japan,*Department of congenital abnormality,National Childrens Medical Research Center, 35-31,Taishido 3-Chome,Setagaya-ku,Tokyo 154,Japan

Restriction fragment length polymorphisms(RFLP) of P450c11 gene were studied among the Japanese Population using P450c11 cDNA clone as a probe.Genomic DNAs from 60 unrelated Japanese individuals were analyzed by Southern blot hybridization.Two RFLPs were detected in Msp I digests of the DNAs.One(A) was characterized by polymorphic bands at 3.4 and 2.5 kilobase-pairs(kbp) and the other(B) was by polymorphic bands at 1.7 and 1.2 kbp.In A RFLP,17 of the 60 persons (28%) were homozygous for the 3.4 kbp band,7(12%) for the 2.5 kbp band and 36(60%) were heterozygous for both bands.In B RFLP,50 of the 60 persons(83%) were homozygous for the 1.2 kbp band,10(17%) were heterozygous for the 1.7 and 1.2 kbp bands but homozygotes of the 1.7 kbp band were not detected.And the third RFLP characterized by polymorphic bands at 5.8 and 4.0 kbp was detected in Pvu II digests.Of the 60 persons,44(73%) were homozygous for the 5.8 kbp band,16(27%) were heterozygous for both bands but no homozygotes of the 4.0 kbp band were detected.These polymorphisms were different from previously reported Caucasian RFLP.

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PRENATAL DIAGNOSIS OF CONGENITAL LIPOID ADRENAL HYPERPLASIA (LIPOID CAH). Z. Klonari, W. Miller, A. Fleischer, C. Abrams, P. Saenger, Dept. Peds., A. Einstein Coll. Med., Bronx, N.Y., Univ. of Calif., San Francisco, Ca., Schneider Children's Hosp., New Hyde Park, N.Y.

Inability to convert cholesterol to pregnenolone causes lipoid CAH, the most severe form of CAH. We now report the prenatal diagnosis of lipoid CAH in a previously evaluated Korean family (JCI 88:1955, 1991). Steroidal responses to ACTH stimulation in both obligatory heterozygous parents were indistinguishable from normal data (JCEM 73:679, 1991). In two pregnancies after diagnosis of the affected proband one male fetus was diagnosed ultrasonographically as unaffected and confirmed postnatally by endocrine testing, and a second XY male fetus lacked sonographically detectable genitalia. Amniotic fluid steroids were low but not absent (Table). The fetus was aborted at 18 weeks. The fetal adrenals were of normal size but contained lipoid droplets; the testes were histologically normal.

Steroid (ng/dl)	Affected fetus (18 wks)	Unaffected Sib (16 wks)	Nl Male Values (mean 16-18 wks)
Preg	99	319	--
Prog	1369	2735	2400
17OH Prog	Nil	156	180
17OH Preg	Nil	205	200
DHEA	Nil	19	21
Δ4-A'dione	14	70	80
Estriol	1.2	80	130
Cortisol	140	2000	1500

Cord blood, E_1, E_2 and E_3 in the affected sib were 106,71, & 9.0 ng/dl, respectively (normal for E_1:900-4000, E_2:200-1600, E_3:1200-15000).

In summary: these data indicate that prenatal diagnosis of lipoid CAH can be made readily, and that maternally derived steroids (particularly Prog) play an essential role in carrying such pregnancies to term.

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PHYSICAL AND ENDOCRINE FINDINGS IN CUSHING'S DISEASE OF CHILDREN TREATED BY TRANSSPENOIDAL OPERATION

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Symptoms of Cushing's disease are developping faster and more characteristically in children than in older patients. This study analizes physical and endocrine findings establishing the diagnosis of pituitary ACTH producing adenomas in 34 children (21 girls and 13 boys). They were operated by transsphenoidal route (successful in 31 children) during 1980 and 1992 at an age of 14.3+/-2.9 (SD),range: 6-18 years. - Unexplained weight gain (60.3+/-17.8,r:35-102 kg, SDS for CA +0.9 +/-1,2) was more frequently complained than growth retardation (148.8+/-13.8,r:119-176 cm, SDS for CA -2.2 +/-2.7), although decreased height SDS and low height velocity (2.2+/-1.9 cm/y) was calculated in all but 3 children. Minimal (164+/-103 ug/l) and maximal plasma cortisol (337+/-153 ug/l) were elevated as well as corresponding ACTH levels (61+/-43 and 123+/-82 ng/l, respectively). CRF stimulated cortisol and ACTH was 429 +/-243 ug/l and 300+/-301 ng/l, respectively in all except 2 cases. Dexamethasone-test and free urine cortisol were less informative.- Conclusion: Impaired growth, increased weight gain, elevated basal and stimulated cortisol and ACTH plasma levels are the most reliable findings in Cushing's disease during childhood.

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HOMOZYGOUS DELETION OF AMINO ACIDS 487-489 IN P450c17 CAUSES SEVERE 17α-HYDROXYLASE (17-OH) DEFICIENCY. Carlos E. Fardella, Linhua Zhang, Pat Mahachoklertwattana, Dong Lin and Walter L. Miller. Dept. of Pediatrics, University of California, San Francisco, 94143.

17-OH and 17,20 lyase activities are mediated by cytochrome P450c17. 17-OH deficiency is rare and only a few cases have been investigated at a molecular genetic level. A 14 y. o. female from rural Thailand was seen by one of us (PM) for fever and muscle weakness for 2 days. Birth and development were unremarkable. Findings included BP 170/120, pre-pubertal breasts, genitalia and axillae and symmetric muscle weakness. Laboratory findings included: Na 149, K 2.1, Cl 100, CO_2 30, BUN 6, cortisol 1.0 μg/dl, LH 94 mIU/ml, FSH 99 mIU/L, undetectable aldosterone, testosterone and estradiol and 46XX karyotype. Ultrasonography showed a prepubertal uterus with normal adrenals and kidneys. A 4-day IM ACTH test showed undetectable 24 h pregnenetriol, 0.9→0.4 mg/24 h pregnenediol, 1.4→3.0 mg/24 h 17KS and 7.6→8.0 mg/24 h 17OHCS. To prove the diagnosis of 17-OH deficiency, the patient's P450c17 gene was amplified and sequenced by our PCR tactic (Lin *et al* J Biol Chem 266:15992, 1991). All sequencing was normal except for a 9 bp deletion in exon 8 that deletes the codons for residues 487-489 of P450c17. This deletion creates a *BclI* site (underlined) in the mutant allele that is

Leu	Ile	Asp	Ser	Phe	Lys
CTG	ATC	GAG	TCT	TTC	AAA

absent in the normal, permitting the distinction between homo- and heterozygosity. PCR followed by *BclI* digestion showed the patient's lesion was homozygous, while the mother and two of three siblings were heterozygous, showing proper Mendelian segregation. This is the first report of this genetic lesion in 17-OH deficiency and the first report of any lesion in a Southeast Asian population.

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ABUNDANT ADRENAL-SPECIFIC TRANSCRIPTION OF THE P450c21A PSEUDOGENE—IMPLICATIONS FOR CONGENITAL ADRENAL HYPERPLASIA. James D. Burgos, Stephen E. Gitelman, Meng Kian Tee, and Walter L. Miller Dept. of Pediatrics, University of California, San Francisco, 94143.

21-hydroxylase is encoded by the 21B gene in the HLA locus on chromosome 6. The duplicated 21A gene is generally regarded as being a non-functional pseudogene, because it has several mutations that would prevent it from encoding P450c21 protein, and because others have reported that it is not transcribed into RNA. Through the use of cDNA cloning and sequencing, RNA-based PCR, and RNase protection experiments, we now show that the 21A and 21B genes are actively transcribed into a family of four large, stable, previously undescribed adrenal-specific mRNAs. These transcripts, termed "Y" RNAs, do not encode P450c21. YA transcripts use the 21A promoter and cap site, and encompass all of the coding region plus the first two introns of 21A. The short YA RNA (3.0 kb) is spliced from a point in the 3' untranslated region of 21A to another three exons beginning 4.5 kb downstream; the long YA RNA (7.5 kb) retains this 4.5 kb, which comprises all of the opposite strand of the XA gene. The short and long forms of YB arise from the 21B promoter in the same fashion. We designed a riboprobe spanning the splices 4.5 kb XA region of the short YA and extending 3' past the A/B gene duplication junction, permitting the display of the four Y RNAs and 21B RNA as five discrete products in an RNase protection assay showing that the long forms of YA and YB predominate over the short forms, and that these are about 20% of the abundance of 21B mRNA. RNase protection experiments also show that expression of Y RNAs is confined to the adrenal. The 21/X/Y system is the first reported example of three overlapping genes in a eukaryotic organism. Transcription of YA exposes 21A gene sequences to single-stranded nicking, possibly explaining the very high incidence of 21A/21B gene conversions that cause 85% of cases of congenital adrenal hyperplasia.