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Determination of 14 steroid hormones in amniotic fluid (AF) : its usefulness in the antenatal diagnosis of 21-hydroxylase deficiency.

In 63 AF of normal pregnancies (14-20 wks), AF levels of testosterone (T), androstenedione (Δ4) were higher in males, while those of pregnenolone (Preg), 17OH-pregnenolone (17OH-preg), 17OH-progesterone (OHP), DHA, Δ5-androstenediol and 17β -estradiol were slightly but significantly higher in females. There was no sex difference in AF levels of preg. sulfate (PS) or DHA sulfate, progesterone, estrone, cortisol and cortisone. Among 4 pregnancies at risk for congenital adrenal hyperplasia (CAH) due to 21-hydroxylase defect, antenatal diagnosis (Dgs) of CAH was made in 1, on the high AF levels of OHP and confirmed at birth. We then investigated whether estimating AF levels of other steroids could consolidate the Dgs. In the AF of the affected girl, T, Δ4 were also drastically elevated, PS, preg and 17OHpreg were just above normal limits (NL), while the other hormones were normal. Similarly T, Δ4 and OHP were drastically elevated in the peripheral blood at birth. In contrast none of the estimations in the cord blood would have made the dgs with certainty, although OHP was above normal. In conclusion the simultaneous measurement of T, Δ4 and OHP may prove to be safer for the definitive antenatal dgs of CAH than single hormone analysis.

(ng/dl)	AF : CAH	NL (♀)	Periph. blood : CAH	NL (♀)
T	25	(3.4-7.2)	419	(30-61)
Δ4	581	(25-60)	2685	(110-237)
OHP	835	(98-149)	16230	(305-973)

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Misleading prediction from fetal HLA typing in the prenatal diagnosis of 21 hydroxylase deficiency adrenal hyperplasia (21 OH DAH

Fetal HLA typing was performed in a 13 weeks pregnant woman for the prenatal diagnosis of 21 OH DAH. She was the mother of an 11 year old girl with late-onset 21 OH DAH (first clinical manifestations appeared at the age of 9 years). HLA typing of the father, mother and child were respectively A3B7/A9B17, A2B5/A2BW38 and A3B7/A2B5. The fetal HLA typing was done on amniotic fluid cells and found to be A3B7/A2B5 ie identical to the haplotype of the affected daughter. The parents wished that the pregnancy be terminated if there was any chance for the fetus to be affected. Termination of the pregnancy was done at 17 weeks of gestation despite the fact that amniotic fluid steroid concentrations suggested an unaffected female fetus (ng/dl : 17 hydroxyprogesterone : 156, testosterone : 4.5, Δ4 androstenedione : 78). The autopsy showed a normal female fetus. This painful experience gives support to the individualization of an acquired 21 OH DAH genetically different (and possibly clinically as well : only girls with late-onset virilisation have been reported so far) from the congenital form and indicates that prenatal diagnosis of the affection should not be based upon fetal HLA typing only.

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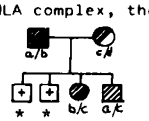
Biochemical evidence of 21-OH deficiency without clinical symptoms in two family members of a CAH-affected child.

HLA typing was performed in a family with a child affected by salt-wasting CAH due to 21-OH deficiency. Father,mother, sister were clinically normal, with absence of signs of virilization or salt-wasting symptoms, normal stature and fertility. Based on the linkage of the gene for 21-OH deficiency to the HLA complex, the sister resulted heterozygous for the gene of CAH:

a)A3,Bw47,Cw6,DRw7 c)A2,B7,DRw2
b)A3,B14,DRw1 d)A1,B8,DRw1
*)died at 3 weeks,with vomiting and diarrhea



Three heterozygous members were therefore found in this family: father,mother,sister. Heterozygous carriers of the CAH-gene are expected to have normal baseline hormone levels.On the contrary father and sister were found to have abnormally elevated hormone levels, even if clinically normal.Both have the CAH gene-linked haplotype ("a"in father, "c"in sister) and the "b" haplotype. It is speculated that the biochemical abnormalities in absence of clinical signs are the result of the combination of a gene for a mild 21-OH defect (linked to the "b" haplotype) with the gene for the severe 21-OH defect (linked to "a" and "c" haplotype).

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An XX male with 21-hydroxylase deficiency.

The patient is the second child of healthy unrelated parents,his older brother is normal. At birth the genitalia were ambiguous; the phallus was adequate in size but there was chordee and perineal hypospadias. The labio-scrotal folds were fused. Gonads were palpable in both groins. The karyotype (blood and skin) was normal female XX. Biopsy of both gonads showed immature testes with no ovarian elements; the XX genotype was confirmed in gonadal tissue. Laparotomy showed normal male and absent female internal genitalia. Hypospadias repair was undertaken and the child was reared in the male sex. At 6 years he was noted to be virilising.He had shown rapid growth and weight gain and development of pubic and axillary hair. Plasma steroids by RIA and urinary steroids by GLC/MS indicated partial 21-hydroxylase deficiency. There was normal suppression with exogenous steroids and a selenocholesterol scan showed bilateral adrenal hyperplasia.He is maintained on hydro-cortisone and continues to make a good progress at the age of 9.This patient has an apparently unique combination of genetic endocrine defects unlikely to be due to chance.It is possible that translocation of part of a Y chromosome has influenced the expression of the 21-hydroxylase locus on chromosome 6.

33 D. BECKER*, M. SALAS*, D. SOBEL*, E. TSALIKIAN*, D. DANEMAN*, A. DRASH. Univ. of Pittsburgh, USA. **Energy Homeostasis in Hypopituitary Children and Controls.**

27 children aged 4-16 yrs with hypopituitarism were studied before (I) and after 6 months of growth hormone therapy (II) and compared with 15 endocrinologically normal children aged 2-12 yrs (8 short stature & 7 suspected hypoglycemia)(III). Plasma glucose (G), insulin (IRI), glucagon (IRG), free fatty acids (FFA) and β-hydroxybutyrate (BOH) were measured serially during a fast and following intravenous glucagon administration which terminated the fast after a maximum of 24 hrs. The mean G nadir during the fast was 51 mg% in I, 44 mg% in II and 44 mg% in III. The glucagon stimulated G rise was significant (35 mg% in I, 33 mg% in II and 35 mg% in III). The FFA concentrations at 0 time were .59, .57 & .67 meq/l in I, II and III respectively. In all 3 groups there was a significant increase of FFA to 1.67, 1.70 & 2.06 meq/l in I, II & III resp. There were no intergroup differences in any of these results. BOH rose equally in groups I & II. There was a significant IRG rise during the fast of 200 pg/ml in I, 197 pg/ml in II & 234 pg/ml in III. Basal IRI values and IRI responses to glucagon were not different in I & II, but significantly higher than III at both times. The higher IRI values in I & II reflect the frequently associated obesity, particularly in older children. We conclude that energy homeostasis in hypopituitarism during fasting is associated with appropriate fat mobilization, ketone production and IRG responses. In 4 patients in I & 2 in III who became hypoglycemic (G<40 mg%) responses were similar.

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Impaired response to Growth Hormone treatment in two subjects with anti-GH antibodies: role of GH aggregates.

Subjects affected by pituitary dwarfism and treated with Growth Hormone (GH) preparations extracted according to the Raben procedure have a high incidence of anti-GH antibodies. These antibodies were detected in 7 out of 22 patients treated with the GRORM (Serono) clinical preparation. Two of them showed a significantly impaired growth rate (19 and 28% less than the mean value in the other 20 patients). Their sera bound labelled GH with a high affinity (K_{aff} 0.68 and 1.16 x 10^{-9}M). The binding capacity was 0.32 and 0.75 mgGH/1, respectively. We fractionated the GRORM preparation by gel filtration (Sephadex G-100) and tested the two sera with different protein peaks. The fraction having a m.w.of 80,000, a K_{av} of 0.20 and an immunoreactivity equal to 84% of the GH monomer was bound more and with a higher association rate. This fraction is 12-24% of the GRORM preparation and it probably consists of GH aggregates.

The present data suggests a major role of the GH aggregates in eliciting anti-GH antibodies production in these patients.