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B.Haglund*, H.Luthman*, E.M.Ritzen
Department of Pediatrics and Clinical Genetics, Karolinska hospital, Stockholm, Sweden.

DNA HYBRIDIZATION ANALYSIS OF 21-HYDROXYLASE DEFICIENCY

To study the genetic defects in 21-hydroxylase deficiency (21-OH def) we analyzed the DNA from 50 deficient children by Southern blotting. Genomic DNA was digested with the the restriction enzyme Taq 1. The cleaved DNA's were fractionated by agarose gel electrophoresis, transferred to nitrocellulose membranes and hybridized with nick-translated DNA containing a near full length copy of the human 21-hydroxylase gene (kindly provided by Dr Perrin White, NY). The hybridized DNA fragments were visualized by autoradiography.

In the normal population two bands of equal intensity were displayed, which migrated as 3.7-Kb and 3.2-Kb bands. It has been shown previously that the two bands represent the two genes for 21-hydroxylase, with the 3.7-Kb band representing the normally transcribed gene. DNA from 28 of the children with 21-OH-def. showed this hybridization pattern. However, in 20 of the cases the intensity of the 3.7-Kb band was only half that of the 3.2-Kb band, and in two of the cases the 3.7-Kb band was absent, These data indicate that the genetic defects in these 22 children are deletions of a major part of the transcribed 21-hydroxylase gene on one or both chromosomes. The two children who have lost the 3.7-Kb band totally are both affected by the salt-wasting form of 21-OH def. Otherwise, no correlation between the different forms of the disease and the hybridization patterns was found.

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Y. Morel*, M. Auvinet*, J. Goetz*, G. Hauptmann*, H. Betuel*, M. David*, J. André* (Introd. by J.Bertrand).
INSERM-U34, Dpt. of Pediatrics, Hôpital Debrousse, Lyon ; CTS de Lyon et de Strasbourg, France.

RESTRICTION MAPPING OF 21-HYDROXYLASE (21-OH) GENES IN CONGENITAL ADRENAL HYPERPLASIA (C.A.H.)

C.A.H. due to 21-OH deficiency is an inherited recessive disorder linked to the HLA-B locus on the chromosome 6. In human beings, there are two 21-OH genes (A and B) located near C4 genes in the major histocompatibility complex ; the nature of the gene alterations responsible of C.A.H. remains matter for debate. Thus restriction maps, C4 and HLA typings were performed in 14 families with 21-OH deficiency. This report is focused on the analysis of TaqI restriction DNA fragments in using 2.7kb murine 21-OH probe including the 3' non-coding region (Amor et al., Proc. Natl. Acad. Sci. USA, 1985, 82, 4453). Four bands were shown : 3.7 and 2.8 kb (gene A), 3.2 and 2.6 kb (gene B). Three series of results were observed : 1) all 4 bands had the same intensity in 9 families in which 2 patients were heterozygous for HLA Bw47. 2) The relative intensity of gene B bands was decreased in patients of 4 families, 2 of which were heterozygous for HLA Bw47 ; a recombination was demonstrated. 3) Gene A bands were missing in index patient and his mother who were heterozygous for HLA B8, a rare haplotype in this C.A.H. Conclusion : 1) in patients of only 5 out of 14 families TaqI restriction maps were modified and a polymorphism cannot be excluded in 2 of them. 2) C.A.Hs with or without salt loss were found in the two first groups selected above and 3) additional analyses are necessary to determine whether each clinical type of 21-OH deficiency is due to a specific 21-OH gene alteration.

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G.Rumsby[1]*, D.B.Grant
Departments of Clinical Biochemistry[1] and Endocrinology, The Hospital for Sick Children/Institute of Child Health, 30 Guilford St., London WC1N 1EH, UK

DELETION OF THE 21-HYDROXYLASE GENE IN CONGENITAL ADRENAL HYPERPLASIA

Congenital adrenal hyperplasia due to 21-hydroxylase deficiency is an autosomal recessive disorder with an incidence of 1:5000 to 1:15000. There are now known to be two 21-hydroxylase genes (21A and 21B) located at the 3' ends of the genes coding for the fourth component of complement (C4) on the short arm of chromosome six. Although the 21A and 21B genes share a high degree of homology and will hybridise to the same DNA probe, they can be distinguished by a number of restriction fragment length polymorphisms.

We have analysed DNA from 30 patients with 21-hydroxylase deficiency - 24 salt losers, 2 non-salt losers and 4 late onset. DNA was isolated from leucocytes, digested with various restriction enzymes and Southern blots prepared. These were hybridised with probes specific for either C4 or the 21-hydroxylase gene. Only two patients were found to have a deletion of the 21B gene on both chromosomes. In one case the adjacent C4B gene was also deleted and appeared to have arisen by recombination between homologous regions of 21A and 21B. 12 other patients were heterozygous for this deletion as judged by the intensity of hybridisation. This finding was not associated with any particular clinical phenotype. A number of cases had no obvious deletion. These may contain only a small change in DNA e.g. a point mutation and are currently being investigated by sequencing of the 21-hydroxylase genes.

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M Dumic*, MI New, S Drucker*, Dept Ped, Univ Hosp Rebro, Zagreb, Yugoslavia; NY Hosp-Cornell Med Ctr, NYC USA

SLAVIC NONCLASSICAL 21-OH DEFICIENCY: A DIFFERENT MUTATION

Nonclassical adrenal hyperplasia due to 21-hydroxylase deficiency (NC21OHD) has been described to occur in high frequency among certain ethnic groups, among them Ashkenazi Jews (1/27), Hispanics (1/53), Yugoslavs (1/63), and Italians (1/333). The haplotype segment HLA-B14;DR1 is in strong linkage disequilibrium with the gene for NC21OHD. Differential ethnic group associations have been described. While B14;DR1 is frequent in nonclassical subjects of Ashkenazi Jewish, Hispanic, and Italian origin, this haplotype is much less frequently observed in Yugoslav and northern European Caucasians. It is postulated that the B14;DR1-associated mutation differs from the non-B14 associated mutation and would produce a difference in clinical and/or hormonal phenotypes. We have observed 10 Yugoslav patients with NC21OHD from 9 unrelated families. Three presented with precocious adrenarche. The remainder, identified through family studies of patients previously diagnosed with CAH or NC21OHD, were noted to be asymptomatic. The clinical presentation among the Yugoslavs was different from that in 84 observed patients who were Ashkenazi Jews, Hispanics or Italians. In the latter group the prominent symptoms were hirsutism in 21, amenorrhea or irregular menses in 9, infertility in 4, acne in 8, clitoromegaly in 3, hair loss in 1, accelerated growth in 1, precocious adrenarche in 20, and 31 were asymptomatic. In these patients with NC21OHD, the B14;DR1 segment occurred in 91% of the non-slavic haplotypes. Only 3 of the Yugoslav patients were found to carry the B14;DR1 haplotype and in each case B14;DR1 was associated with Italian, Hungarian, or German ancestry. In summary, we propose that the Yugoslav mutation in NC21OHD is different from other European ethnic groups.

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D. Konitsiotou*, Chr. Papasteriadou*, E. Spanos*, C. Dacou-Voutetakis.
First Department of Pediatrics, Athens University, and Evangelismos General Hospital Athens, Greece.

HLA ANTIGENS IN GREEK FAMILIES WITH CONGENITAL ADRENAL HYPERPLASIA (CAH).

Data on HLA typing in patients with CAH have indicated an association of certain forms of CAH to specific HLA antigens. We looked for HLA ABC antigens in 136 subjects belonging to 41 families, of hellenic origin, with one or two members affected with CAH, and in 380 apparently normal individuals who served as controls. For the calculations only one affected member from each family was inserted. We found the HLA B14 antigen in 42.8% of cases with the latent form of CAH (LF) versus 5% of the controls (p 0.001). The HLA B7 antigen was found in 23% of subjects with the salt wasting form (SW) versus 7.6% of the controls (p 0.05). The A3 antigen was found in 41% in SW versus 17% of the controls (p 0.02). All indiviluals with SW and HLA B7 antigen have A3B7 haplotype. No other antigens showed an association to any form of the disease. More specifically the HLA B5 antigen, found by others in increased frequency in the simple virilizing form of CAH, was not found increased in our group (35.5% in the affected versus 33.1% in the controls). Thus while the B14 antigen seems to be associated with a specific form of CAH in individuals of different ethnic and geographical background, this is not so with other CAH associated HLA antigens. The interpretation of this heterogenuity is only speculative at present.

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C.Broessler*, B.Horak*, K.Lichtwald*, D. Schoenberg, P.Vecsei* Dep.Pharmacol.and Dep. Pediatr.Endocrinol., Univ.Heidelberg, FRG

SCREENING FOR CONGENITAL ADRENAL HYPERPLASIA (CAH):COMBINED RADIOIMMUNOASSAY (RIA) OF 17-HYDROXYPROGESTERONE AND 21-DESOXYCORTISOL IN DRIED BLOOD SPOTS WITHOUT SOLVENT EXTRACTION.

The extraction step with diethyl ether for a specific RIA of 17-hydroxyprogesterone (17-OHP) in dried blood spots (PANG et al JCEM 45,1003,1977) is unpracticable for mass screening of CAH. Based on the observation that 21-desoxycortisol (21-DOF) is elevated in untreated CAH (FRANKS JCEM 39,1099,1974) we designed a RIA intentionally with an antiserum (AS) crossreacting with 17-OHP (100%) and 21-DOF (48%). Dried blood spots on filter paper of 4,25 mm diameter - approx. 10 µl whole blood - were eluated with buffer containing chloral hydrate to separate the steroids from protein binding, and incubated with 1,2,6,7-H^3-17-OHP and AS 1: 3000 in microtiter plates shaking at room temperature for 21 hours. With a standard curve of 0 - 10.000 pg 17-OHP/spot we found for 17-OHP/21-DOF calculated in ng/ml plasma: sensitivity 3; coeff.var. at level 100 interassay 9%, intraassay 5%; in 2.274 newborns at day 5 mean 133,24; +/- 2 SD 36,56 - 229,92; in 2 untreated CAH range 1.000 - 2.000. In 15 adults the coeff.corr. between 17-OHP/21-DOF in dried blood and both steroids determined separately in plasma with chromatographic purification was 0,89 (linear), in 8 children with CAH treated or untreated it was 0,79 (exponential).