

704

CENTRAL NERVOUS SYSTEM CYSTINE STORAGE IN CYSTINOSIS. Adam J. Jonas, Rebecca Speller, Susan B. Conley and *Harvey Rosenberg. University of Texas Medical School, Houston, Departments of Pediatrics and *Pathology (Spon. by Wallace Gleason).

Cystinosis is a disorder of lysosomal transport that is characterized by the lysosomal storage of the amino acid cystine. The disorder causes progressive renal failure and may damage other organs such as the thyroid, retina, and pancreas. Central nervous system function is generally thought to be normal in this disorder. We measured central nervous system cystine in autopsy specimens obtained from a 25 year old woman with nephropathic cystinosis who had a history of cerebral atrophy and short term memory loss. The dura, anterior pituitary, and choroid plexus had marked cystine storage with levels that were 450, 810, and 4,600 times those of controls, respectively. These levels approached 1 $\mu\text{mol}/\text{mg}$ protein and were comparable to hepatic and conjunctival cystine levels. The frontal lobes, corpus callosum, and cerebellum had the least storage with cystine levels that were 30-40 fold elevated over controls. All areas of the brain that were sampled had evidence of involvement including the midbrain, basal ganglia, medulla, pons, and spinal cord. While this patient's central nervous system problems cannot be attributed to cystinosis alone, the biochemical findings raise the question of eventual central nervous system dysfunction in cystinosis.

CORRELATION OF OVARIAN FUNCTION WITH GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE LEVELS IN GALACTOSEMIA. Francine R. Kaufman, Won G. Ng, George N. Donnell, Univ. of So. Cal. School of Med., Childrens Hosp. of Los Angeles, Dept. of Peds., Los Angeles, CA.

Galactosemia (G) is due to either partial or complete deficiency of the enzyme galactose-1-phosphate uridyl transferase (Ts). In females with G, there is a high incidence of premature ovarian failure. This study attempts to correlate the level of Ts activity in erythrocytes with ovarian function. In 28 patients (pts), ages 1-32 yrs, Ts by microassay technique, LH, FSH and estradiol levels were measured. Stimulation with gonadotropin-releasing hormone (GnRH) was performed to assess ovarian function in pts with basal LH and FSH levels <50 mIU/ml (18 pts). Ts activity was found to be zero (normal >7.0 units) in 25 pts; 23 of these had evidence of ovarian insufficiency. In pts >13 yrs, there was a progressive rise of the gonadotropin levels as the ovary failed, although in 1 pt this occurred at 26 yrs of age after the birth of a normal infant. In pts <12 yrs, elevated basal FSH levels and an exaggerated gonadotropin response to GnRH was seen. Three pts, ages 30, 26, 24 yrs, had minimal Ts activity detected by microassay (0.15, 0.018, 0.004 units, respectively). These pts all had evidence of normal gonadal function by either normal response to GnRH stimulation in 2 pts and repeated fertility in 1 pt. CONCLUSION: Ovarian damage in G occurs in the complete absence of the Ts enzyme and minimal levels of Ts appear to be enough to preserve ovarian function.

706

AMINO ACID (AA) AND CSF NEUROTRANSMITTER (NT) PROFILES IN NEONATES WITH GALACTOSEMIA (GAL). Mark Korson, Mira Irone, Harvey L. Levy. Harvard Med Sch, Children's Hosp, Depts of Pediatrics and Neurology, State Lab Inst, Boston, MA.

GAL is an inborn error of galactose metabolism characterized by CNS and liver disease, cataracts and hyperaminoaciduria. The pathogenesis of cellular damage in GAL has not been defined; AA disturbances may play a role. We have studied AA in blood and CSF and the CSF AA-related NT metabolites in neonates with GAL, and have related the results to the other biochemical and the clinical disturbances. Twenty-two infants had blood AA determinations from the routine newborn screening specimen. Substantial elevations of tyrosine and phenylalanine were present in 3/5 who later became septic. Plasma AA were measured in 11/22 infants immediately prior to therapy. Tyrosine was the most conspicuously elevated (211-1515 μM ; nl 70 + 29 μM). Less markedly elevated AA included phenylalanine, threonine, serine, glycine, alanine, ornithine, lysine and histidine. Methionine was only mildly increased, while the branched-chain AA were normal. CSF AA in 5 infants reflected plasma abnormalities. There was no correlation between the degree of AA abnormality and either the severity of liver disease or the presence of cataracts. However, 2 variant infants with residual transferase activity who were phenotypically normal had normal AA profiles. CSF NT metabolites were normal in 5 infants.

There appears to be a pattern of AA abnormality characteristic of neonates with classic GAL. The early CNS effect in GAL, however, seems not to be associated with AA-related NT deficiencies.

707

ABNORMAL TAQ I SITE IN THE THIRD EXON OF THE APOA1 GENE IN A FAMILY WITH LOW APOA1 LEVELS AND PREMATURE CORONARY ARTERY DISEASE. J. A. Ladias¹, P. O. Kwiterovich¹, H. Smith¹, S. K. Karathanasis², S. E. Antonarakis¹. ¹The Johns Hopkins Univ. Sch. Medicine, Dept. Pediatrics, Baltimore, ²Harvard Medical School, Dept. Cardiology, Boston.

In order to identify families with premature atherosclerosis due to single gene defects, DNA from about 100 such patients has been screened for mutations in the Apolipoprotein gene cluster ApoA1-ApoC3-ApoA4. One patient, 35 years old with chest pain and low levels of ApoA1 (90 mg/dl), has been found who had an absence of a Taq I restriction site in the third exon (codons 33-34) of the ApoA1 gene.

The abnormal Taq I site was present in his father who also had low ApoA1 levels and myocardial infarction at age 34 and in his younger sister who did not have low levels of ApoA1. DNA polymorphic haplotype analysis for the ApoA1-ApoC3-ApoA4 gene cluster indicated that the sister and the proband had inherited different ApoA1 alleles from their mother who had hyperalphalipoproteinemia, indicating that the sister might have inherited a "hyperalpha" allele from the mother.

Although the exact nucleotide change has not yet been determined, the most common Taq I site mutations involve CG to TG or CA substitutions and in the abnormal ApoA1 gene in this family may lead to nonsense or a missense mutation Arg-Gln at codon 34.

708

GENE TRANSFER INTO PRIMARY HEPATOCYTES FOR SOMATIC GENE THERAPY. Fred D. Ledley, Gretchen Darlington, Tina Hahn, Savio L.C. Woo (Spon. by Arthur Beaudet). Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, Texas.

Somatic gene therapy of many inborn errors of metabolism may require placement of recombinant genes into liver cells in order to provide substrates and cofactors for holoenzyme activity. We report retroviral mediated transfer of recombinant genes into isolated mouse hepatocytes. Hepatocytes isolated from young mice and cultured in defined, serum-free media (SUM-3) exhibited growth (3-5 divisions) and differentiated hepatic function for 1-2 weeks in culture. Cells were infected with a recombinant retrovirus carrying the neomycin (G418) resistance gene, selected with G418 and analyzed *in situ*. No uninfected hepatocytes survived in 250 $\mu\text{g}/\text{ml}$ G418. Infected cells expressing the recombinant neomycin resistance gene survived selection in G418, exhibited characteristic hepatocyte morphology, and continued to express liver specific genes alpha₁-antitrypsin and phenylalanine hydroxylase. These data indicated that primary hepatocytes had been successfully transfected with the recombinant gene and expressed the recombinant gene product. Hepatocytes containing recombinant genes can be transplanted into host animals and will continue to express differentiated function. These experiments provide a model for future gene replacement therapy in which recombinant genes may be introduced into autologous hepatocytes and transplanted into patients in order to reconstitute a defective biological function and reverse the process of genetic disease.

709

A NEW FORM OF GLUTARIC ACIDEMIA TYPE II (GA2). James P. Loehr, Frank E. Frerman, Stephen I. Goodman. Univ. Colorado Sch. Med. Department of Pediatrics, Denver

GA2 may be due to inherited deficiency of electron transfer flavoprotein (ETF) or ETF-ubiquinone oxidoreductase (ETF-QO), proteins that transfer electrons from soluble mitochondrial flavoprotein dehydrogenases to the main respiratory chain. Cell lines from 11 severely affected GA2 infants were assayed for ETF by acyl CoA:Q1 activity (using purified general acyl CoA dehydrogenase and ETF-QO as electron donor and acceptor), and for ETF-QO by comproportionation of ETF and by NADH:ETF reductase activity.

All but one were deficient in ETF (5 patients; none with anomalies) or ETF-QO (5 patients; all with anomalies). The one that was not was from a girl with nonketotic hypoglycemia, a typical Zellweger facies, normal serum very long chain fatty acids, and glutaric, ethylmalonic, 3-hydroxyisovaleric and isovalerylglutamate in urine who died at 6 months of age. Autopsy showed hypertrophic cardiomyopathy, moderate lipid deposition and canalicular stasis in liver, focal glomerular immaturity and cortical cysts in the kidney, and cysts in the cerebral cortex and basal ganglia. Glutaryl CoA dehydrogenase activity in fibroblasts was normal.

Specific Activity (nmol/min/mg protein)

	ETF	ETF-QO (comprop)	ETF-QO (NADH red)
Patient	1.72	12.9	1.45
Controls	.73±.20(n=11)	15.4±3.6(n=11)	1.86±0.15(n=6)

This GA2 patient may have a defect in electron transfer between ETF-QO and complex III, and her phenotype may indicate interaction between peroxisomal and mitochondrial compartments.