61

PRENATAL DIAGNOSIS OF MEVALONIC ACIDURIA BY STABLE ISOTOPE DILUTION GCMS. Hoffmann, G., Gibson, K.M., Nyhan, W.L. and Sweetman, L. (introduced by C. Bachmann). Dept. of Pediatrics, Univ. Calif. San Diego, La Jolla, CA, USA.

Prenatal diagnosis of the organic acidurias that are life threatening or cause mental retardation is an important part of genetic counseling and may permit prenatal therapy. Eight different organoacidopathies have been successfully diagnosed prenatally by stable isotope dilution GCMS assays; there are 15 others potentially diagnosable. This methodology has been applied to mevalonic aciduria, the first documented inherited disorder of cholesterol and nonsterol isoprene biosynthesis in man. In a mother at risk for this disease the urinary excretion of mevalonic acid at 16 weeks of pregnancy of 5.6 mmol/mol creatinine was 35 times the mean normal level (range 0.09 - 0.22 mmol/mol creatinine, 5 control females). The analysis of amniotic fluid at this time indicated a 3000-fold elevation of mevalonic acid (240 µmol/l; range in 4 control amniotic fluids: 0.054 - 0.11 µmol/l), indicating the presence of an affected fetus. The diagnosis was confirmed by demonstration of deficient mevalonate kinase in amniocytes and ultimately in liver from the abortus. Mevalonic acid was found to be highly elevated in the abortus tissues. Concentrations ranged from 840 to 1120 µmol/kg in adrenals, gonads, liver, lymph nodes and spleen. An even higher level was detected in the brain, where the concentration was 1810 µmol/kg (control < 1 µmol/kg). This may reflect a particular need for cholesterol in brain development.and is consistent with the severe developmental delay of the index patient with mevalonic aciduria. After termination of the pregnancy the mother's urinary excretion of mevalonic acid fell to 0.20 mmol/mol creatinine, within the normal range.

62

MEVALONIC ACIDURIA. MEMBRANE COMPOSITION AND FLUIDITY IN AN INBORN ERROR OF CHOLESTEROL SYNTHESIS. Hübner C, Hoffmann G, Kohlschütter A, Hermanussen M, Gibson KM, Sweetman L. University of Hamburg and University of California San Diego, Depts. of Pediatrics, Hamburg, FRG and La Jolla, USA.

Mevalonate aciduria is an inherited disorder of cholesterol synthesis. Mevalonate kinase activity in lysates of fibroblasts from a patient was <1% of control mean and cholesterol synthesis in intact fibroblasts with labeled acetate or mevalonolactone was 33-54% of parallel controls. However, fibroblast growth rates in media containing whole serum and lipoprotein-deficient serum (LPDS), respectively, were normal. Mutant fibroblasts grown in the presence of whole serum had a normal cholesterol content and a normal free to esterified cholesterol ratio. Lipid fluidity of plasma membranes of fibroblasts was measured by steady-state fluorescence polarization. Fluorophores used were diphenylhexatriene (DPH), a cationic analogue (TMA-DPH), and a set of anthroyloxy fatty acids. For all fluorophores used the results for the mutant cells were within the normal range. Even when grown in LPDS medium, membrane fluidity of mutant cells was not increased. Hence, mutant cells were able to preserve a normal growth rate and membrane function despite their impaired cholesterol synthesis. We are now investigating the compensatory mechanisms developed by cells with this mutation. (Supported by DFG grant Ko756.)

63

PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS BY LINKED DNA-PROBES Buys CHCM (1), ten Kate LP (1), Penninga D (1), Farrall M (2), Williamson R (2), Dijkstra I (1), and te Meerman GJ (1), Depts Human Genetics, State Univ. Groningen (1) and Biochemistry, St. Mary's Hosp. Med. Sch., London

Several groups recently reported DNA markers on chromosome 7 in linkage with the locus of the cystic fibrosis (CF) mutation. From the linkage data collected to date probes pJ3.11 and met appear to be sufficiently close to the CF locus (each with about 1% of recombination in approx. 400 meioses studied) to be used in diagnosis. They can be applied in prenatal diagnosis and carrier detection in informative families with an affected child. As an illustration we present the first diagnosis on CF by DNA markers carried out prenatally in the Netherlands. In the family involved one parent appeared to be heterozygous for met D and the other for pJ3.11, both on TaqI-restricted DNA. Their affected child appeared to be homozygous for both probes, whereas DNA isolated from a chorionic villus biopsy at 11 weeks pregnancy showed heterozygosity for both pJ3.11 and met D. Our risk calculations assuming a 3% recombination frequency between 3.11 and the CF locus, and a 4% between met and CF, resulted in a greater than 99.5% probability for the foetus to be non-affected.

64

ASSAY OF AMYLO-1,6-GLUCOSIDASE-TRANSFERASE ACTIVITY BY RELEASE OF GLUCOSE FROM PHOSPHORYLASE LIMIT DEXTRIN - A REASSESSMENT. Gutman, A., Ben-Bassat. Y., Schramm, H., Lilling,S., Dept. Biochemistry, Hadassah University Hospital, Jerusalem, Israel.

The assay of glycogen debranching activity by measurement of the release of glucose from phosphorylase limit dextrin (PLD), was originally used for assay of enzyme activity in muscle and liver tissue. Results are corrected for the presence of nonspecific glucosidases by subtraction of the activity obtained with glycogen as substrate. When assays are performed on muscle or liver, the activity obtained with glycogen is very low and its exact nature is not of practical importance. The widespread use of blood cells and fibroblasts, in which the activity on glycogen is considerable, has prompted an examination of the apparent kinetic constants of this reaction. In liver and muscle the apparent Km for PLD ranged from 0.5-2.0 mg/ml, whereas that for glycogen was lower by an order of magnitude. The Vmax with glycogen as substrate did not exceed 20% of that with PLD. In leukocytes and platelets the Km for glycogen was higher than for PLD (0.1 and 0.5 mg/ml for PLD, 0.4 and 0.8 mg/ml for glycogen, in platelets and leukocytes, respectively), and the Vmax for PLD exceeded that for glycogen by 80%. In fibroblasts the Km for both PLD and glycogen was 1.5-3 mg/ml and the differences in Vmax were small. These results indicate that substrate concentration should be varied according to the kinetic constants of each cell type and point to the importance of distinguishing between the (low?) activity of the debranching enzyme on glycogen and nonspecific hydrolysis.

65

HEXOSEMONOPHOSPHATE (HMP) SHUNT ACTIVITY AND GLUCOSE TRANSPORT IN POLYMORPHONUCLEAR (PMNL) AND LYMPHOCYTE CELLS OF PATIENTS WITH GLYCOGENOSIS (GSD) I. Bashan, N., Hagay, Y., Potashnik, R. and Moses, S.W. Faculty of Health Sciences, Ben-Gurion University of the Negev, and Pediatric Laboratory, Soroka Medical Center, Beer Sheva, Israel.

GSD I is characterized by deficiency of liver glucose-6-phosphatase (Ia) or of a microsomal transporter for G6P (Ib). Both variants present with similar clinical features; however, GSD Ib patients suffer from neutropenia and impaired functions of their PMNL cells. This study measures HMP shunt activity and glucose transport in PMNL cells and lymphocytes of patients with GSD Ia and Ib, as well as of controls. The HMP shunt activity decreased significantly in intact PMNL cells of GSD Ib patients as compared to GSD Ia patients and to controls (Ib: 15.5±2.5, Ia: 47, control: 44.7±5.0 nmole/mg prot/h; P control:Ib<0.001). The reduced HMP shunt activity rose to above normal levels in disrupted GSD Ib PMNL cells (Ib: 117±18, control 75±10; P<0.005). HMP shunt activity of intact lymphocytes was the same in all 3 groups studied. The rate of deoxyglucose transport into GSD Ib PMNL cells was 30% of normal (0.86±0.38 as compared to control of 3.1±0.7 nmole/mg prot/min). This abnormal transport was present neither in GSD Ib lymphocytes nor in GSD Ia PMNL cells and lymphocytes. The striking limitation of glucose transport via the cell membrane of PMNL cells of GSD Ib patients can account for the impairment of leucocyte function which is characteristic of GSD Ib but not found in GSD Ia.

66

THE NATURAL HISTORY OF LIVER GLYCOGENOSIS DUE TO PHOSPHORYLASE KINASE OR PHOSPHORYLASE DEFICIENCY: A LONGITUDINAL STUDY OF 45 PATIENTS. Willems PJ[1], Berger R[1], Gerver WJ[2] and Fernandes J[1]. Departments of Pediatrics, University of Groningen[1] and Limburg[2], The Netherlands.

The breakdown of glycogen is catalysed by the (glucagon triggered) activation of the phosphorylase(P)-phosphorylase kinase (PK) system. Deficiencies in either of these enzymes leads to glycogen storage disease (GSD). In this study we report on the course of this type of GSD in 41 male patients with X-linked liver PK deficiency and 4 patients with autosomal liver P deficiency. PK deficiency was established in erythrocytes (0.4±0.2 U/min.gr Hb vs 5.5±2.0 for controls) and in white blood cells (0.08±0.04 U/min.mg protein vs 0.56±0.19 for controls). P deficiency was established in liver biopsy specimen (7.1-15.0 nmoles/min.mg protein vs 12.1-60.0 for controls). Tentative differentiation between liver PK and P deficiency can be performed by a glucagon provocation test. In liver PK deficiency, a normal response of blood glucose was observed (from 3.5±0.6 to 7.2±1.1 mmol/l, n=17) while in liver P deficiency this rise was subnormal (from 3.8±1.1 to 4.5±0.9 mmol/l, n=3). The majority of both PK and P deficient patients presented with severe hepatomegaly (93%), growth retardation (68%), delayed motor development (52%), hyperlipidemia (75%), fasting hyperketosis (44%), and elevation of glutamate pyruvate transaminase (57%). With age, these clinical and biochemical abnormalities gradually disappeared and adult patients were in good health in spite of persisting enzyme deficiency.