559

POLYAMINE (PA) METABOLISM IN CYSTIC FIBROSIS (CF). M.G. Rosenblum, R.C. Beckerman, L.M. Taussig, B.G.M. Durie, B.H. Bowman, D. Barnett, D.H. Russell. Univ. of Arizona Health Sciences Center, Tucson, AZ. and University of Texas Medical Branch, Galveston, TX.

Prior studies have shown that PA levels are elevated in blood components of CF homozygotes. We have studied urinary PA levels and ^{14}C spermidine metabolism in controls and CF patients. The urinary PA levels in 7 CF homozygotes were 2-10 fold higher than in 8 heterozygotes and 6 normals ($p<0.0001$). No statistically significant differences were found between heterozygotes and controls. The ^{14}C spermidine plasma decay curves in two CF patients with severe clinical disease (NIH Score <50) were not significantly different from normal. However, urinary excertion of the ^{14}C radiolabel by the 2 CF patients was only about 10% as compared to 60-76% excreted by normals after 72 hours. Urine samples were obtained and NIH Clinical Scores were assigned to a group of 12 CF patients. Those with scores <70 (N = 4) demonstrated statistically significant lower levels of putrescine ($p<0.05$) and significantly higher levels of spermine ($p<0.01$) than those with scores >70 (N = 8). These data show that although plasma decay curves for ^{14}C spermidine are similar to normals, the urinary excretion pattern suggests sequestration in CF patients with severe clinical disease. Further, polyamine levels are elevated in the urine of CF homozygotes and appear to correlate well with the patient's clinical status.

Supported by USPHS Grants CA-14783 and CA-17094 from the National Cancer Institute (D.H.R.). R.B. is an ALA Fellow.

560

STUDIES OF H-Y ANTIGEN IN A 46,XYp- PHENOTYPIC FEMALE WITH TURNER STIGMATA AND UNDIFFERENTIATED GONADS. Ron Rosenfeld, Luigi Luzzatti, Raymond Hintz, Orlando J. Miller, Gloria Koo, Stephen Wachtel; Stanford University Dept of Peds, Stanford; Depts. Hum. Gen/Dev & OB/GYN, College of Physicians & Surgeons, NYC; Mem. Sloan-Kettering Cancer Center, NYC.

H-Y antigen is believed to be the product of testis-determining gene(s) on the Y chromosome. A case of 46,XYp- phenotypic female provided an opportunity to evaluate both sexual and somatic determinants on the Y chromosome. At birth, the infant had lymphedema of the extremities, increased nuchal skin folds and normal female external genitalia. Q and C banding of peripheral leukocytes demonstrated a 46,XYp- karyotype. Laparotomy demonstrated bilateral 1.5cm gonads with normal uterus and tubes. Gonadal sections revealed fibrous stroma with focal aggregations of undifferentiated cells arranged in cords or clusters. At 8 years she is a phenotypic female with height in the 25th %, multiple stigmata of Turner Syndrome and elevated gonadotropins. H-Y phenotype assignment is based on ability of test cells to adsorb H-Y antibodies from mouse H-Y antisera, which are then reacted with mouse sperm. Skin fibroblasts of the patient failed to adsorb H-Y antibodies; her peripheral leukocytes adsorbed considerably less than her father's. She was thus typed H-Y$^{\pm}$, a phenotype consistent with a deletion model in which a majority of H-Y gene copies has been lost. Demonstration of the structural deletion of the Y chromosome supports the hypothesis that loci exist on the short arm of the Y which determine H-Y antigen, testicular differentiation of the primitive gonad and suppression of somatic stigmata of Turner Syndrome.

561

ISOELECTRIC FOCUSING OF α-L-FUCOSIDASE FROM CYSTIC FIBROSIS AND CONTROL SKIN FIBROBLASTS. Thomas F. Scanlin and Mary Catherine Glick. (Spon. by Stanton Segal). School of Medicine, University of Pennsylvania, Children's Hospital of Philadelphia, Department of Pediatrics, Philadelphia.

α-L-Fucosidase activity is elevated in cystic fibrosis (CF) skin fibroblasts while nine other acid hydrolases including neuraminidase have activities similar to those in the control fibroblasts (Biochem. Biophys. Res. Commun. 79,869,1977). Extracts of skin fibroblasts from CF individuals and age, sex, and race matched controls were analyzed by isoelectric focusing on thin layer polyacrylamide gels to determine if the elevated activity of α-L-fucosidase in the CF cells resulted from a difference in a specific isoenzyme. Fibroblasts were seeded at a density of 3.0×10^6 cells/150 cm^2 and harvested after 7 days with trypsin. Cell pellets were suspended in Triton X-100, broken in a Dounce homogenizer and centrifuged at 10,400g for 20 min. The supernatant solutions were electrofocused for 2 h in an LKB Multiphor apparatus both before and after treatment with Vibrio cholerae neuraminidase. Two separate ampholine gradients, pH 3.5-10 and pH 4-7, were used. The gels were cut in 2 mm slices and α-L-fucosidase activity was assayed using 4-methylumbelliferyl-α-L-fucopyranoside as substrate in citrate buffer, pH 5.8. The CF and control fibroblasts had similar isoenzyme patterns under all of the conditions described with the exception that several CF preparations showed a predominance of the isoenzymes with higher isoelectric points. USPHS GM07025 and The National Foundation-March of Dimes.

562

FAILURE OF ASCORBIC ACID (AA) THERAPY IN NEPHROPATHIC CYSTINOSIS. J.A. Schneider, J.J. Schlesselman, S.A. Mendoza, S. Orloff, A.D. Godfrey, J.D. Schulman. Dept. of Ped., Univ. of Ca., San Diego and NICHD, Bethesda, Md.

Since there is no specific therapy for cystinosis and since AA decreases the free-cystine content of cystinotic fibroblasts (Science 86:1040, 1974), a randomized double-blind trial of high dose (200 mg/Kg/day) AA was undertaken. An unblinded committee monitored the data every 4 months. At entry the AA and placebo (PL) groups were comparable in all major clinical parameters. There were 52 patient years for the AA group and 50 patient years for the PL group. Of the 11 patients who died, began chronic dialysis or were transplanted, 8 had received AA. Neither treatment affected the WBC cystine content. Patients receiving AA had a greater mean rise in serum creatinine (Cr). This was especially true in patients whose initial Cr was ≥ 1.0 & <3.5. Although a small benificial effect of AA could not be excluded statistically, it was more likely that AA hastened the progression of renal failure. In view of this adverse risk-benefit ratio the study was terminiated.

Months in Study*	Rise in Cr (mg/dl) AA	PL	p
	(All Patients)		
12 mo	.82±.29(18)**	.42±.20(19)	<.3
16 mo	1.30±.43(16)	.58±.26(14)	<.2
20 mo	2.78±.81(12)	.97±.38(9)	<.1
	(initial Cr of ≥ 1.0 & <3.5)		
12 mo	.90±.30(11)	.29±.13(12)	<.1
16 mo	1.66±.62(9)	.40±.15(10)	<.1
20 mo	3.16±.98(9)	.84±.23(6)	<.1

*All patients at 20 mo. are included in 16 mo. data, etc. **mean ±SEM(n)

563

ATYPICAL PRESENTATION OF TRISOMY 13 MOSAICISM Robin Slover, Eva Sujansky, Arthur Robinson. Department of Biophysics and Genetics, University of Colorado Medical Center and National Jewish Hospital, Denver.

We have seen two unrelated cases of Trisomy 13 mosaicism with the main presenting symptom of severe bilateral deafness and microtia respectively. To our knowledge such a presentation of Trisomy 13 mosaicism has not been reported previously. Although the clinical symptoms of Trisomy 13 mosaics are variable, mental retardation and cleft palate are the most frequent findings. The first case was a 10 year old mentally retarded male with severe bilateral deafness, duplication of the ureters and a port wine nevus. The second case presented with a right sided microtia, a heart murmur and bilateral simian creases. His psychomotor development at 6 months was normal. Chromosome analysis from the peripheral blood lymphocytes culture revealed in both cases Trisomy 13 in 15% of cells. Our experience indicates that the diagnosis of Trisomy 13 mosaicism should be considered in children with deafness or microtia asscciated with minor malformations.

564

TREATMENT OF GLUTATHIONE SYNTHETASE DEFICIENT FIBROBLASTS BY INHIBITION OF γ-GLUTAMYL TRANSPEPTIDASE WITH SERINE-BORATE. Stephen P. Spielberg, Jean deB. Butler, and Joseph D. Schulman, Depts. of Pediatrics and Pharmacology, Johns Hopkins Univ., Baltimore, and NICHD, Bethesda.

Glutathione synthetase (GSH-S) deficiency (5-oxoprolinuria) results in decreased cellular glutathione (GSH) content (10-20% of normal), and secondary over-production of 5-oxoproline. γ-glutamyl transpeptidase (GGTP) is the primary catabolic enzyme for GSH, and inhibition of this enzyme might thus be an approach to correcting the consequences of GSH-S deficiency. L-serine inhibits fibroblast GGTP. Inhibition is markedly enhanced by sodium borate buffer, 20 mM serine-20 mM borate causing >95% inhibition. Serine-borate added to Eagle's MEM produced a dose and time dependent increase in GSH content of GSH-S deficient cultured fibroblasts. GSH content was doubled at 24 hours with 40 mM serine-40 mM borate. Borate alone was without effect. Conversion of ^{14}C-glutamic acid to 5-oxoproline by GSH-S deficient cells was decreased by 70% to near normal levels by 24-hour pre-treatment with 40 mM serine-borate. The increased cell GSH content may block overproduction of 5-oxoproline from excess γ-glutamylcysteine by feed-back inhibiting γ-glutamylcysteine synthetase. Treatment produced no apparent toxicity; cell amino acid concentrations were unaffected other than an increase in serine and phosphoserine. The study demonstrates the possible therapeutic value of an inhibitor of a major catabolic enzyme for a substrate decreased secondary to a deficiency of its synthetic enzyme.