● **738** STUDIES OF A NEW GENETIC DISEASE CHARACTERIZED BY A DEFICIENT PMN LEUKOCYTE SURFACE GLYCOPROTEIN(S). D. Anderson, F. Schmalstieg, W. Shearer, M. Tosi, B. Hughes, S. Kohl, & T. Springer. Baylor College of Medicine (Houston), Univ. of Texas (Houston & Galveston), & Harvard (Boston).

Four unrelated pts (3 F, 1 M) with granulocytosis, delayed umbilical cord severance, periodontitis, &/or recurrent soft tissue infections were identified. Adhesion-dependent PMN functions (chemotaxis, adherence-spreading, aggregation, phagocytosis, orientation, & antibody-dependent cellular cytotoxicity) were significantly ($p < .001$ for each assay) diminished in each pt. All fathers, mothers or siblings (2 M, 1 F) were asymptomatic & demonstrated normal PMN functions. PMN homogenates of each patient demonstrated a severe deficiency (<5% normal) of a high M.W. glycoprotein (GP138) (SDS-PAGE). A NaB^3H_4 labelling technique indicated that GP138 represents a major surface GP complex of normal PMNs (11.4±7 µg/mg total protein), but was undetectable on the surface of pt PMNs. GP138 content of maternal or female sibling PMNs was diminished (mean = 3.3 µg/mg), but was normal (mean = 13.6 µg/mg) in paternal or male sibling suspensions. Monoclonal antibodies directed at the α & common β subunits of 3 leukocyte "adhesive" GPs (OKM1, LFA-1, P150,95) were employed to demonstrate the absence of each of these GPs on the surface of patient PMNs & diminished (10-50% normal) surface OKM1 & β on maternal or female siblings' PMNs (flow microfluorimetry). Thus, GP138 deficiency syndrome represents a new disorder(s) related to the absence of critical adhesive PMN GPs. Since males & females are affected & the carrier state can be detected only in females, its mode of genetic transmission is uncertain.

739 **RING 21 CHROMOSOME: LOCALIZATION OF THE BREAKPOINTS WITHIN A 2 KB DNA FRAGMENT. Stylianos E. Antonarakis, James F. Gusella, Gail Stetten, Michael J. Potter, Paul C. Watkins, Haig H. Kazazian, Jr. Johns Hopkins University School of Medicine, Dept. Pediatrics, Baltimore, Harvard Medical School, Massachusetts General Hospital, Genetics Unit, Boston and Integrated Genetics Inc., Framingham, MA.**

In order to understand the mechanism of chromosomal breakage at the DNA level, we recently studied a patient with a de novo ring 21 chromosome. His karyotype was described as 45, XY, -21/46, XY, r(21) (p13q22). Thus, he is monosomic for the sub band 21q22.3 in every cell and trisomic for the remainder of chromosome 21 in at least 70% of his cells.

Using five single copy DNA fragments randomly isolated from chromosome 21 as probes, we have found the following: (i) Four of DNA fragments mapped to the trisomic portion of chromosome 21. (ii) Using DNA polymorphisms associated with these fragments, we determined that the ring chromosome had originated from the mother. (iii) One DNA fragment (pPW231C) mapped at the breakpoints of the r(21). This latter conclusion was based on the fact that abnormal fragments were present in Southern blots of DNA derived from the proband's leukocytes and fibroblasts and absent from parental DNA. Using several restriction digests we were able to localize the breakpoints to within a 2.1 Kb EcoRI DNA genomic fragment. Nucleotide sequencing of both the normal and the rearranged DNA will provide important information concerning the DNA sequences involved in the rearrangement leading to formation of the ring 21 chromosome.

† **740** SPONTANEOUS REVERSION AT THE HPRT LOCUS IN CELLS FROM A PATIENT WITH A MILD VARIANT OF THE LESCH-NYHAN SYNDROME. Arthur S. Aylsworth, Laird G. Jackson, Nicholas M. Kredich. Univ. of North Carolina Dept.of Pediatrics and the Biological Sciences Research Ctr, Chapel Hill; Jefferson Medical College Div. of Genetics, Philadelphia, Pa.; Duke Univ. Dept. of Medicine and the Howard Hughes Medical Institute, Durham, N.C.

We have studied cells from a patient who was followed with a diagnosis of spastic cerebral palsy until the age of 9 years when he was found to have a deficiency of hypoxanthine phosphoribosyltransferase (HPRT). He has dysarthria, choreathetoid movements, and an IQ in the normal range. He is nonambulatory because of the spasticity and has not engaged in self-mutilation other than some scratching and nail biting. Autoradiography of his cultured skin fibroblasts after exposure to ^{3}H-hypoxanthine revealed a frequency of $HPRT^+$ cells in the range of 10^{-5} to 10^{-4}. His cells were cloned in 6-thioguanine and then grown in the absence of selective medium. The frequency of $HPRT^+$ cells ranged from 10^{-6} to 3×10^{-5} after approximately 23 cell doublings. Cells exposed to ^{3}H-hypoxanthine in the presence of aminopterin showed significant low level uptake in all cells indicating that these cells all contain some HPRT activity.

Cells were grown in HAT medium and clones of HAT-resistant cells were isolated. A sensitive HPLC assay for HPRT has been developed to study enzyme activity in these cells. The milder phenotype of the patient may be related to cells that are spontaneously reverting to $HPRT^+$ as well as to the low background level of HPRT in all cells.

† **741** A TWO HOUR PHENYLALANINE (P) LOAD FOR CARRIER DETECTION OF PHENYLKETONURIA (PKU). Joan E. Bailey-Wilson, Miriam G. Blitzer, Grace Kissling, Emmanuel Shapira. Tulane University School of Medicine, The Hayward Genetics Center, and Louisiana State University School of Medicine, Department of Biometry, New Orleans.

Since the enzymatic determination of P-hydroxylase activity remains limited to liver tissue, the diagnosis of most PKU homozygotes and especially heterozygotes relies on P-loading tests. In the present study, plasma was obtained before a P load (t=0) and a various time intervals after the load. The P and T concentrations in plasma were determined using a Beckman 6300 Amino Acid Analyzer. Twenty-five controls, 20 obligate heterozygotes and 10 patients with PKU were studied. The most informative parameters were obtained from values at t=0, 60 and 90 minutes. Age, weight, the use of birth control pills, values of P, T, P/T, P/T^2, P/T^3, the natural log of these parameters, and their various transformations were subjected to discriminant analysis with the statistical program BMDP7M. The various ratios for PKU homozygotes were 1-2 orders of magnitude higher than those of controls and heterozygotes. The analysis at t=0 (as suggested by some authors) revealed a 14.8% misclassification for heterozygotes. The best discrimination using 1 blood sample was at 60 minutes; only 1 of the controls was misclassified (3.7%). When females on birth control pills were excluded and using the variables of age, P/T at t=0 and 60 minutes, the function correctly classified all individuals. This procedure enabled the best discrimination between heterozygotes and controls thus far described.

● **742** TRANS-ACTING REGULATION AND ALTERNATIVE RNA SPLICING FOR THE ARGININOSUCCINATE SYNTHETASE (AS) LOCUS. Arthur L. Beaudet, Svend O. Freytag, Tsung-Sheng Su, and William E. O'Brien. Baylor College of Medicine, Department of Pediatrics, Houston.

We are attempting to delineate the molecular basis for both metabolite regulation of AS and enzyme overproduction in canavanine resistant (Can^r) cells. We isolated normal and Can^r variant lymphoblast cell lines from the parent of a citrullinemia patient. These cells contain one normal gene and one mutant allele producing an abnormal mRNA which is distinguishable by S1 nuclease analysis. Activities for AS (nmol/min/mg protein) were as follows: lymphoblasts in arginine medium <0.01; lymphoblasts in citrulline medium 0.05; three Can^r lymphoblast variants 0.79-1.2. S1 nuclease analysis demonstrated parallel increases in both normal and abnormal RNA in the Can^r cells in proportion to the increase in enzyme activity. Since selective pressure in the Can^r isolation is restricted to the functional allele, the increased expression of the mutant allele must reflect a trans-acting mechanism affecting the expression of both alleles. We also have detected alternative RNA splicing involving the presence or absence of an exon (probably the 2nd exon) in the 5′ untranslated region of the mRNA. This exon is present in only a very minor proportion of mRNA from human liver or fibroblasts, but is present in a larger proportion of mRNA from Can^r cells or baboon liver. The functional significance of this result is unclear at present.

† **743** L-CARNITINE FOR CYSTIC FIBROSIS. Paul J. Benke, Budge McKey and Jose Foradada. University of Miami School of Medicine, Mailman Center and Dept. of Pediatrics, Miami, FL 33101

A fatty acid abnormality has been suggested for cystic fibrosis (CF) because CF secretions have 1) excess fatty acids bound to glycoprotein and 2) high levels of leukotrienes, a specific group of fatty acids. Carnitine transports fatty acids into mitochondria where they are oxidized. Serum free and acyl carnitines in 10 CF patients averaged 46.27 and 6.35 nmol/ml respectively, and could not be distinguished from control levels. However, the basis for the research was that excess carnitine may increase pulmonary metabolism of fatty acids. We have given oral carnitine to 3 CF patients with recent clinical deterioration and 6 stable CF patients.

Sick patients with inspissated secretions and recent decreased pulmonary function showed greatest improvement in pulmonary function (FEV 1 and FVC) 6-15 days after beginning 3-4 grams of carnitine per day. Improvement was dramatic (40-100%). Bronchospasm improved in the 2 patients who demonstrated it. Positive effects were not permanent, however, and pulmonary function began to deteriorate in 2 of 3 patients after 6 weeks of therapy.

Pulmonary function did not improve significantly in 5 of 6 older, stable patients with cystic fibrosis when 3-4 gram/day of carnitine was given for 3 weeks. However, all patients had loosening of secretions and 4 of 6 reported improved ability to cough. Although sweat sodium appeared to decrease in 4 of 6 patients, the results were variable and no consistent pattern was observed.

These studies suggest that L-carnitine may have useful short term effects in acutely ill patients with CF.