● 780 COMPLEMENTATION IN VARIANTS OF SULFITE OXIDASE DEFICIENCY. Vivian E. Shih, Roseann Mandell, Jean L. Johnson, Harvard Medical School, Massachusetts General Hospital, Department of Neurology, Boston and Duke University Medical Center, Department of Biochemistry, Durham, N.C.

Hereditary disorders causing a deficiency of sulfite oxidase (SO) activity result in the accumulation of sulfite, sulfocysteine and thiosulfate and a clinical syndrome of seizures, developmental delay, and ectopia lentis. The defect may be in the structural enzyme protein (apoenzyme) of SO or due to the absence of its active molybdenum (Mo) cofactor, in which case Mo-dependent xanthine oxidase activity is also reduced. Study of fibroblast lines of one patient (A) with SO apoenzyme defect and 5 unrelated patients (B-F) with Mo cofactor deficiency revealed no detectable SO activity in any case. Complementation tests showed that SO activity was restored when Line B was cocultured with each of 5 other lines. No activity was detected in other pairwise combinations. Complementation occurred without heterokaryon formation, suggesting that the corrective factor may be a diffusible precursor of the cofactor. These findings confirm heterogeneity of SO deficiency; at least 3 variants can be distinguished. Line A has a mutation of the structural gene for SO apoenzyme which may have affected the binding of the heme or Mo cofactor but is without effect on its antigenicity. Among the cofactor deficient lines, Line B clearly has a defect different from and correctable by the other 4 lines. These 4 could have a common defect or non-complementing lesions in the biosynthetic pathway of the cofactor.

● 781 MOLECULAR HETEROGENEITY OF THE ARGININOSUCCINASE (ASAL) MONOMER FROM ASAL-DEFICIENT FIBROBLASTS L. Simard, W.E. O'Brien, R.R. McInnes (spon. by J. Clarke) Res. Inst. Hosp. for Sick Children, Dept. Genetics, Toronto, Ont., Baylor College of Med., Dept. of Ped., Houston, Texas

Complementation analysis of the urea cycle disorder ASAL deficiency has shown it to be characterized by striking genetic heterogeneity due to multiple alleles at a single locus, possibly the ASAL structural gene. We have examined the fibroblast ASAL monomer from 28 patients using denaturing polyacrylamide gel electrophoresis and protein blotting. Liver extracts and nine control fibroblast strains had a single protein band of MW ~49,500 which cross-reacted with an anti-ASAL antisera. All 28 mutant strains had immunologically cross-reactive material (CRM) in the 49,500 region varying from trace to normal levels. Five mutants with the largest amount of 49,500 MW CRM also had the highest residual enzyme activity or the greatest ability to complement other mutants. In contrast to controls 27 of the 28 mutants had substantial lower MW CRM of 42,000, 38,000, 35,000, 34,000 and 30,500. In addition to the 49,500 MW band, several of these bands were also prominent in the blots of two obligate heterozygote strains. Only trace amounts of the lower MW bands were seen intermittently in controls. One mutant had unique bands of MW 39,000 and 25,000 suggesting the presence of a truncated polypeptide. These studies provide clear evidence of extensive heterogeneity in the abundance of the ASAL monomer (MW 49,500) in the mutant strains, and supporting evidence that ASAL deficiency affects the ASAL structural gene, resulting in the production of ASAL monomers with increased in vivo lability.

● 782 DEFECTIVE CREATINE METABOLISM IN GYRATE ATROPHY OF THE CHOROID AND RETINA (GA). I. Sipila, D. Valle, S. Brusilow and M. Kaiser-Kupfer. Johns Hopkins Univ Medical Sch, Dept Pediatrics, Balto; NIH, NEI, Bethesda.

GA patients exhibit chorioretinal degeneration and histologic muscle abnormalities. Owing to an inherited deficiency of ornithine aminotransferase, they accumulate ornithine to levels which are 10x normal and well above the Ki of glycine transamidinase, the first enzyme in the creatine biosynthetic pathway. Previous work utilizing an insensitive assay has shown reduced levels of the creatine (cr) presursor guanidinoacetic acid (gaa) in the urine of patients with GA (Sipila et al, J.Clin.Invest. 67: 1805,1981). We developed a specific HPLC/fluorometric method for measuring these compounds in biologic samples. The lower limit of detectability for cr is 0.1 pmol and for gaa 3 pmol. We found subnormal levels of both cr and gaa in patients with GA.

	Plasma (uM)*			Urine (umol/m^2/d)**		
	gaa	cr	(n)	gaa	cr	(n)
control	2.2±1.0	25.4±27.5	(35)	259(116-913)	356(51-1441)	(9)
GA	0.6±0.3	6.1± 7	(14)	19(1-61)	121(1-355)	(6)

* mean ± 2 SD; ** mean (range)

In addition, we found reduced levels of total cr in adult GA skeletal muscle: patients (n=3) mean (range) is 6.5 nmol/mg prot (5.1-7.8); control (n=3) 31.4 (23.5-42.0). We conclude from the reduced levels of gaa that cr synthesis is impaired in GA and that this eventually leads to reduction of tissue levels of cr despite normal dietary intake. These results support the hypothesis that cr deficiency is an important pathophysiologic factor in GA.

† 783 THE ROLE OF ATP IN LYSOSOMAL CYSTINE EFFLUX. M.L. Smith, R. Potashnik, A.A. Greene, A.J. Jonas and J.A. Schneider. UCSD, La Jolla, CA, Dept. of Pediatrics.

In cystinosis there is a defect in cystine efflux from the lysosomes which is expressed in both cultured fibroblasts and E.B. virus transformed lymphoblasts. Cystine efflux from lysosomes isolated in Hepes/Sucrose from control lymphoblasts is stimulated by the addition of MgATP, while efflux from lysosomes isolated from control fibroblasts is not. To clarify the role of ATP and the lysosomal ATPase in cystine efflux, we determined the pH of lysosomes isolated in simultaneous experiments from both cell types by measuring the fluorescence of intralysosomal FITC-dextran. The pH of lymphoblast lysosomes is 6.5±.05. This is lowered to 6.2±.03 by the addition of MgATP to the incubation buffer. Fibroblast lysosomes have a pH of 5.5±.07 which is not altered by the addition of MgATP. However, if the pH of fibroblast lysosomes is raised by preincubation with KCl, ATP causes acidification to near-initial values. In both cell types, there is no significant difference between cystinotic and control lysosomes in initial pH, ability to use ATP to produce an acidic pH, or the amount of proton-translocating ATPase activity. Our interpretation of these data is that cystine efflux from lysosomes requires a highly acidic intralysosomal pH. Since fibroblast lysosomes isolated in Hepes/Sucrose have a pH of 5.5 independent of ATP addition, cystine efflux is not stimulated by ATP in this system. In contrast, lymphoblast lysosomes are acidified in the presence of ATP, explaining the requirement of cystine efflux for ATP.

● 784 ACHONDROPLASIA DUE TO DNA INSERTION INTO THE TYPE II COLLAGEN GENE. Charles. M. Strom (Spon. by Lawrence M. Gartner) University of Chicago, Pritzker School of Medicine, Department of Pediatrics, Chicago, IL 60637

Achondroplastic dwarfism is an autosomal dominant disorder in which 90% of cases represent new mutations. The molecular mechanisms of the disruption of normal cartilage and endochondral bone formation remain unelucidated. Type II collagen is the major collagen of cartilage and plays an important role in stabilizing the extracellular matrix. In this study, fibroblast DNA from a patient with classical sporadic achondroplasia and peripheral white cell DNA from her parents with normal stature were analyzed. Southern filters were prepared following BamHI digestion and were probed with pgHCol(II)al, a probe to the carboxypropeptide and triple helical region of the human type II procollagen gene (Strom, C. and Upholt, W.B., Nucleic Acids Res., in press). The DNA from both parents showed only the normal 4.2kb band. The achondroplastic DNA, however, showed a faint band at 4.2kb and a denser band at 6.2kb. Further analysis revealed that the polymorphic 6.2kb band is due to an insertion of DNA sequences complementary to the pgHCol(II)al probe near the 3' end of the type II collagen gene. This is the first demonstration of type II collagen gene changes in the human chondrodystrophies.

● 785 GENETIC LINKAGE STUDIES IN THE MARFAN SYNDROME Petros Tsipouras, Clair Francomano, Reed E. Pyeritz, Ann Pizanis, Richard Mulroney, John A. Phillips III, Francesco Ramirez. Departments of Biochemistry, Pediatrics and OB-Gynecology, Rutgers Medical School, and Pediatrics and Internal Medicine, Johns Hopkins University. (Spons by C.R. Scott)

In order to define a single major gene defect which could account for the Marfan syndrome (MS) phenotype we used a high frequency EcoRI restriction fragment length polymorphism in the pro 2(I) gene of type I collagen as a marker for genetic linkage studies. The presence or absence of one EcoRI recognition site creates a two allele system observed after hybridization with a 6.75kb pro 2(I) genomic probe (Tsipouras et al. JCI 72:1262, 1983). We studied affected and unaffected individuals in three families with MS. In family A the segregation of the clinical phenotype was concordant with the segregation of the marker allele, although random cosegregation could not be excluded due to the small number of informative matings. In family B the segregation of the clinical phenotype and marker allele was discordant. Family C was not informative due to lack of heterozygosity for the marker allele. Our data suggest the some variants of MS may be linked to mutations in or near the pro 2(I) collagen gene. This is in agreement with the observation that a structural alteration in the pro 2(I) chain of type I collagen results in the MS in one patient (PNAS 78:7745, 1981). Our data further suggest that MS is biochemically heterogeneous and that defects in more than one gene may result in the phenotype.