716 TREATMENT OF ISOVALERIC ACIDEMIA WITH GLYCINE SUPPLEMENTS. Mary C. Naglak, Kevin M. Madsen, Phillip P. Dembure, Rino Salvo, Louis J. Elsas. Emory Univ. Department of Pediatrics, Division of Medical Genetics, Atlanta, Georgia.

Isovaleric Acidemia is treated with L-leucine restriction and supplemental glycine. Added glycine augments conversion of isovaleric acid (IVA) to isovalerylglycine (IVG) through glycine-N-acylase. The optimum glycine dosage for management of isovaleric acidemia is not known. In this study we evaluate a 9 year old female whose cultured fibroblasts have 4% of control isovaleryl-CoA dehydrogenase activity. Glycine supplements between 0 and 600 mg/kg/d were compared at weekly intervals with restricted leucine intake (54 mg/kg/d) and after acute loading with leucine (120 mg/kg). Under restricted leucine intake IVG excretion rose from 12.3 to 33.6 mmols/gm creatinine when glycine supplements were increased from 0 to 50 mg/kg/d. As glycine supplements of 100 and 150 mg/kg/d were added, maximum excretion of IVG continued; however, when increased to 300 ($\bar{x}$ plasma gly=978 μM) and 600 mg/kg/d ($\bar{x}$ plasma gly=2547.5 μM) IVG excretion fell to 13.8 and 16.3 mmoles/gm creatinine, respectively (N1 plasma gly=219 μM). When acute leucine loads were compared at glycine supplements of 190 mg/kg/d ($\bar{x}$ plasma gly=424 μM) and 600 mg/kg/d ($\bar{x}$ plasma gly=1636 μM), urinary IVG excretion was 194 and 419 mmoles/gm creatinine, respectively. We conclude that when IVA accumulation is minimal, optimum glycine dosage is < 150 mg/kg/d; under these conditions glycine intakes of 300 and 600 mg/kg/d inhibit IVG production. However, when acute insults raise IVA production glycine supplements of 600 mg/kg/d increase IVG production two fold.

●717 EFFICIENT DETECTION OF Y CHROMOSOMAL DELETIONS, TRANSLOCATIONS AND MOSAICISM USING CHROMOSOME-SPECIFIC DNA PROBES. H. Ostrer, Departments of Pediatrics, Biochemistry and Molecular Biology, University of Florida, Gainesville (Sponsored by E. Ayoub).

We have studied 25 patients with suspected structural abnormalities of the Y chromosome by Southern blot analysis using Y-derived DNA probes. These probes spanned much of the Y chromosome and included: Yp - DXYS5, DXYS1, DXYS25; Y cen - DYZ3; Yq - DXYS15, DYZ1; Yp and Yq - DXYS11, DYS8. Hybridization to the repeated sequence probe, DYZ1, was used to quantify Y chromosomal mosaicism. Three of eight females with gonadal dysgenesis and chromosomal mosaicism were found to have deleted Y chromosomes as the basis for this mosaicism. One of the five females with 46,XY gonadal dysgenesis was found to have a deletion of Yp, encompassing DXYS5 The others appear to have intact Y chromosomes, suggesting autosomal recessive inheritence. The four 46,XX males and the 45,X male were found to have translocations of Yp, including DXYS5. In some instances, these included larger chromosomal segments. The Y chromosomal breakpoints were different in the two females with Y-autosomal translocations. These studies suggest that most Y chromosomal deletions or translocations occur as the result of relatively simple genetic events. Y chromosomal material, when present, was detected with one of three probes,DXYS5 (Yp), DYZ3 (Y cen) and DYZ1 (Yq). Thus, the process of detecting Y chromosomal genetic material can be made very efficient for analyzing unknown patient samples.

●718 DEFECTIVE INTRACELLULAR COPPER TRANSLOCATION IN MENKES KINKY HAIR SYNDROME. Seymour Packman, Sally A. Sample and Susan C. Whitney. University of California, San Francisco, Department of Pediatrics.

Menkes kinky hair syndrome is an X-linked neurodegenerative disorder causing tissue-specific increases in copper (Cu) and metallothionein (MT) concentrations. Previous work has shown that MT gene regulation may be normal in Menkes. However, abnormalities in Cu retention and utilization by mutant cells suggest that the basic defect may reside in the transfer of Cu across cell compartments. We tested this hypothesis in Menkes fibroblasts by examining the distribution of Cu and zinc (Zn) in crude particulate and cytosolic fractions (pellet and supernatant of a 100,000 g-min spin). In control cells, the Cu content of particulate fractions incrementally rose up to 2 mcg/mg cell protein as cytosolic Cu content was increased (up to 1.4 mcg/mg protein) by growth conditions. In contrast, in Menkes cells, over a range of cytosolic Cu concentrations indistinguishable from that in the control cells ($P>0.10$, Kolmogorov-Smirnov test), particulate Cu content remained significantly lower than in controls (± 0.3 mcg/mg protein, $P<0.001$, extended Mantel-Haenszel test). A similar difference in dose-response was observed when 64-Cu accumulation was assessed. At overlapping cytosolic 64-Cu concentrations, 64-Cu appeared in control particulate fractions in significantly higher concentrations than in Menkes cells. In contrast, particulate-cytosol distributions in 65-Zn accumulation studies were identical in Menkes and control. We conclude that a major consequence of the Menkes mutation is a specific failure of Cu translocation across a cell compartment.

●719 HEMOPHILIA A: EXPERIENCE WITH PRENATAL DIAGNOSIS AND CARRIER DETECTION USING DNA ANALYSIS. D.G. Phillips, S.E. Antonarakis, H.H. Kazazian, Jr. The Johns Hopkins Univ. Sch. Medicine, Dept. Pediatrics, Baltimore, MD

Prenatal diagnosis and carrier detection of hemophilia A using DNA analysis has been attempted in 113 families since February 1985. DNA polymorphisms within and linked to the factor VIII (F8) gene were used as markers for the defective F8 gene in these families. When possible, diagnosis was preferentially based on DNA markers within the F8 gene, while in about 25% of families, diagnosis was based on DNA markers linked to the F8 (theoretical error rate in these latter cases is 1-5%). The results of these studies are as follows: Prenatal Diagnosis: 64 families. Diagnoses made: 45 (normal males: 20, affected males: 14, carrier females: 5, noncarrier females: 6). Diagnoses not made: 26 [Because a) the carrier status of a female fetus was not requested, b) pregnancy was terminated prior to testing, or c) family was noninformative in the initial phase of the study because of lack of DNA polymorphism markers]. Carrier testing: 72 families (carriers: 33, noncarriers: 28, noninformative families: 4, new mutations to hemophilia: 5, incomplete linkage analyses: 2).

These data suggest that carrier detection and prenatal diagnosis of hemophilia A using the present DNA polymorphisms is possible in most but not all families at risk. Additional DNA polymorphisms within the F8 gene are needed to further improve the efficacy and eliminate the possibility of error in diagnosis due to the recombination distance of some DNA markers from the F8 gene.

●720 FURTHER CHARACTERIZATION OF LYSOSOMAL SYSTEM y^+, Ronald L. Pisoni, Jess G. Thoene, Rosemary M. Lemons and Halvor N. Christensen. Univ. of Michigan, Depts. of Biol. Chemistry and Pediatrics, Ann Arbor, MI

Using a trans-stimulation property associated with lysine exodus, we have demonstrated previously a system mediating the transport of cationic amino acids across the lysosomal membrane of human fibroblasts. By studying instead the uptake of arginine into highly purified fibroblast lysosomes, obtained by centrifuging through Percoll density gradients, we now examine additional characteristics of this system. For arg uptake it displays a broad pH optimum from pH 7.0-8.0. The rate of arg uptake is 10-fold greater at pH 7.0 than 5.0, thus favoring net entry of arg into lysosomes as a result of the low intralysosomal pH. In contrast, external MgATP accelerates lysosomal efflux of cationic amino acids while inhibiting their influx. Trans-stimulation of arg uptake is seen when lysosomes have been loaded with 2-aminoethyl-L-cysteine. Arg uptake (.03 mM) is strongly inhibited by the L-isomers of external 3.3 mM arg, lys, orn, 2,4-diaminobutyrate, 2-aminoethylcysteine and his, whereas D-arg, neutral and anionic amino acids have little effect. In addition, lysosomal arg uptake is inhibited by α-N-methyl-L-arg (72%) and ε-trimethyl-L-lys (49%), neither of which are recognized by the plasma membrane System y^+. These observations indicate that lysosomal System y^+ is structurally different from System y^+ of the plasma membrane of the human fibroblast and various other cells. Thiocholine (TC) depleted cystinotic fibroblasts of their accumulated cystine to the same level and rate as produced by cysteamine supporting the view that TC may react with cystine to form a mixed disulfide recognized by lysosomal System y^+ similar to the one formed by cysteamine. Support ackn. from Grant AM32281, NIH.

●721 PRENATAL DIAGNOSIS OF SALLA DISEASE (SD) Martin Renlund and Pertti Aula (Spon. by William A. Gahl). Department of Pediatrics and Obstetrics, University of Helsinki, Finland.

Salla disease (SD) is a lysosomal storage disease characterized by severe early onset psychomotor retardation and intralysosomal accumulation of free sialic acid (SA). Four pregnancies at risk for SD were monitored by quantitation of free and total SA in amniocytes and amniotic fluid, using a sensitive HPLC method. Three children were normal. One child was affected. This child was born before SA had been discovered to be the storage compound in SD. Free SA was only slightly elevated in the amniotic supernatant fluid of the affected child (5.7 nmol/ml) as compared to the three unaffected (2.8, 3.3 and 3.5) and controls (1.7 to 5.7) and therefore could not be used for diagnostic purposes. Free SA content of the amniocytes from the affected child was 2.6 nmol/mg protein, which was about 5 times higher than the content in the three unaffected cases (0.3, 0.4 and 0.8, respectively) and 14 controls (0.3-0.9). The ratio of free /total SA of the amniocytes also clearly distinguished the affected pregnancy (0.138) from the unaffected (0.023-0.048) and controls (0.018-0.053). These findings represent the first successful prenatal diagnosis of SD and indicate that both amniocyte free S A and free/total SA ratio should be monitored in pregnancies at risk for SD.