706 STUDIES ON THE FRAGILE X SYNDROME IN SUBJECTS WITH MENTAL RETARDATION OF UNKNOWN ETIOLOGY. Nancy J. Carpenter, Lawrence G. Leichtman and B. Say. Childrens Medical Center, Department of Clinical Genetics, Tulsa, OK.

The fragile X syndrome is a recently described entity comprised of X-linked mental retardation, macro-orchidism and a chromosomal fragile site at Xq27. We have studied 50 institutionalized males and 15 non-institutionalized males and found 6 (9.2%) to have this disorder. The frequency of the fragile site ranged from 8-28%. The fragile site was also detected in 4 female obligate carriers (100%) and in 4 potential carriers (67%). Of the 8 carriers, 4 were mentally retarded. The frequency of the fragile X ranged from 4-26% however there was no correlation with age or IQ. Studies of X-inactivation showed that either the normal or the fragile X were inactive. In 5 of 6 females, the normal X was preferentially inactive. Skin fibroblast cultures from 2 subjects who expressed the fragile site in lymphocytes gave 0% and 1% expression of the chromosomal aberration. The clinical findings of the affected males, one of whom was prepubertal, showed enlarged testicular volumes. Perseverative speech patterns, low IQ's (30-40), and characteristic facial features including prominent supraorbital ridges and prominent chins were noted. We believe the clinical work-up of any mentally retarded male without microcephaly and other significant somatic defects is incomplete unless the exclusion of the fragile X chromosome has been made. We further suggest that females with any degree of mental retardation, especially mild, should be considered for such studies.

707 HOMOCYSTINURIA DUE TO METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR) DEFICIENCY: RESPONSE TO A HIGH-PROTEIN DIET. Stephen D. Cederbaum, Kenneth N.F. Shaw, David R. Cox, Richard W. Erbe, Gerry R. Boss, and Robert E. Carrel; UCLA; Childrens Hospital of Los Angeles; UCSF; and Harvard;Depts. of Psych. and Peds.

M.S., a 5-year-old girl presented at 10 months with developmental delay, severe hypotonia, respiratory difficulty and inability to focus. She was the offspring of a white-black mating but had straight blonde hair and blue eyes. Urine and plasma homocystine (HC) were 90-210 mg/g creat. (nl. not detected), and 0.53 mg/dl (nl.N.D.) respectively, whereas plasma methionine (met) (0.064-0.23) was below or near the lower limits of normal.

There was no fibroblast growth in the met-free, HC supplemented medium and whole cell met biosynthesis was 6% of normal.MTHFR was 2.9 ± 0.2 nmole HCOH/mg prot/hr (n1.9.0 ± 2.0), whereas cystathionine synthase and methionine synthase were normal.

When fed a high-protein diet, plasma and urine HC fell to 0.068 mg/dl and 25-35 mg/g creat. respectively. On lower (normal) protein intake, previous higher values recurred.

Within 3 days of beginning high-protein, muscle tone improved, the patient pulled to standing, focused on and responded to other individuals, and began developmental progress. Within 3 months, the hair grew in curly and dark-brown. She is now moderately retarded, neurologically impaired, but physically quite healthy. The pathogenesis of the neurological disease in this patient appears related to a yet undefined deficiency, overcome, at least in part, by increased protein intake.

708 WILSON'S DISEASE FIBROBLASTS--MUTANTS OF INTRACELLULAR COPPER TRANSPORT. Wai-Yee Chan, Mary A. Coffman and Owen M. Rennert. Department of Pediatrics and Biochemistry and Molecular Biology. University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

Wilson's Disease (WD) and Menkes' Disease (MD) are two disorders of copper (Cu) metabolism. Cultured skin fibroblasts from patients of either disorder demonstrated elevated intracellular Cu. Different abnormal cytosolic Cu binding ligands have been demonstrated in these two types of cells indicating different molecular mechanisms. MD cultured fibroblasts have increased sensitivity to the cytotoxic effects of Cu. WD fibroblasts were cultured in MEM with 10% fetal bovine serum (average Cu content 50.2 ± 20.1 ng/ml). Cu ($CuSO_4$) was added to media to make concentrations of 2,10,15,20,25,30,35 ug/ml. Confluent cultures were incubated in the Cu enriched media for 48 hours. At 15 hours in media with >30 ug Cu/ml, cytotoxic effects of Cu were observed in some cultures. At the end of the experimental period, cultures exposed to <30 ug Cu/ml were morphologically normal, while those incubated with >30 ug Cu/ml exhibited cytotoxicity. This cytotoxic level is comparable to normal cells and higher than that of MD cells (10 to 25 ug Cu/ml). Determination of intracellular Cu content showed that the cytotoxic effect of Cu was observed in WD cells at an intracellular Cu concentration of 2200 ng/mg cell protein. This is comparable to that for normal cultured fibroblasts (1913 ± 608 ng/mg cell protein) and higher than that for MD cells (975 ± 331 ng/mg cell protein). These results show that the block in intracellular Cu transport in WD and MD cells occurs at different sites in the transport chain and further establishes the validity of the WD fibroblast as a model of Wilson's Disease.

709 OUTCOME OF EARLY AND LONG-TERM MANAGEMENT OF CLASSICAL MAPLE SYRUP URINE DISEASE. Carol L. Clow, Theresa M. Reade and Charles R. Scriver. MRC Genetics Group, McGill Univ.-Montreal Children's Hosp. Res. Inst., Montreal and the Réseau Provincial de Médecine Génétique, Québec, Qué.

We describe the outcome of 8400 treatment days in the lives of four classical MSUD patients (present ages: 1 3/12, 5 7/12, 7 1/12 and 8 11/12 yrs). All were diagnosed on clinical signs (vs newborn screening). Acute-phase treatment, beginning on or before the 12th day of life, comprised peritoneal dialysis, intravenous lipid, and early intestinal alimentation. Mean age at discharge from hospital was 29 dy. Parents were taught to monitor urine keto acid excretion (by 2,4-DNPH reaction) and to make up weighed diets. There were only 16 readmissions to hospital for loss of metabolic control (plasma [leu] >1 mM) in the group (89 dys; 1.05% treatment days); serious neurological symptoms were avoided. The group mean plasma leucine level (for levels below 1 mM) during treatment was 0.44 mM (normal for age range, 0.077±0.021 mM; mean and SD); 8.6% of 1042 values exceeded 1 mM during treatment. Group mean plasma valine and isoleucine levels were 60% and 70% of the plasma leucine value. Tolerance for dietary leucine did not exceed 620 mg/dy in any patient. Somatic growth was normal and the mean current IQ/DQ score is 101; the three oldest patients attend normal schools. A characteristic EEG pattern was observed in 3 patients in the acute stage but not during long-term treatment. These results were obtained in an ambulatory program with home visiting.

710 ASSIGNMENT OF THE FIRST HIGHLY POLYMORPHIC DNA MARKER LOCUS TO A HUMAN CHROMOSOME REGION. Bérengère de Martinville, Arlene R. Wyman, Ray White, and Uta Francke, Yale Univ., Dept. Hum. Genet., New Haven, CT; MIT, Dept. Biol., Cambridge, MA; Univ. of Utah, Dept. CVMB, Salt Lake City, Utah.

Recombinant DNA technology has provided a new class of genetic markers that will greatly facilitate the construction of a human gene map (Botstein et al, Am J Hum Genet 32:314, 1980). DNA sequence variation in human populations is detectable as variants in the lengths of the DNA fragments produced by restriction enzymes (Restriction Fragment Length Polymorphism, or RFLP). A cloned recombinant DNA fragment has been found to be homologous to Eco Rl fragments of different length in different individuals. This RFLP locus includes at least 8 alleles that are inherited as codominant traits (Wyman and White, PNAS, 77:6754, 1980).

We used the recombinant plasmid pAW101 as a probe to examine the presence of homologous sequences in the DNA extracted from 17 human x Chinese hamster somatic cell hybrid clones. The hybrids were derived from 5 different human donors, 4 of whom were heterozygous producing 2 band patterns (all different) on Southern blots. The recombinant plasmid probe did not hybridize with DNA from the hamster parental cell line. The presence of human homologous sequences in the hybrids correlated exclusively with the presence of human chromosome 14. Two hybrids contained chromosome 14 in a frequency of greater than 1 per cell and were positive for both alleles. Three hybrids containing only the distal half of the long arm of 14, as part of a translocation, were still positive. These results assign the first highly polymorphic RFLP locus identified in man to region q21→qter of chromosome 14.

711 REGIONAL CHROMOSOMAL ASSIGNMENT OF THE STRUCTURAL GENE FOR HUMAN ACID β-GLUCOSIDASE. Evelyn A. Devine, Moyra Smith, Bridget Shafit-Zagardo, Francisco X. Arredondo-Vega, Robert J. Desnick. Mount Sinai School of Medicine, Division of Medical Genetics, New York, NY.

Human acid β-glucosidase (GBA), the lysosomal enzyme deficient in Gaucher disease, has been mapped to chromosome 1 using somatic cell hybridization techniques. Antibody against purified human GBA was raised in Balb/C mice and a sensitive double antibody immunoprecipitation assay was developed to detect human GBA activity in mouse RAG X human fibroblast hybrids. The mouse anti-human GBA antibody was specific for human GBA and no cross-reactivity between mouse β-glucosidase or several other human enzymes, including neutral β-glucosidase could be detected. Fifty-two primary, secondary and tertiary hybrid lines, derived from three separate fusion experiments, were analyzed for human GBA and enzyme markers for each human chromosome. There was 100% correlation between the presence of human GBA and the presence of human chromosome 1 as determined by cytogenetic and chromosome 1 enzymatic marker (phosphoglucomutase 1 and fumurate hydratase) analyses. All other human chromosomes segregated independently of human GBA. The structural locus for human GBA was further defined to the region 1p11→ 1qter by using a RAG X human fibroblast line which carried a mouse-human rearrangement involving human chromosome 1.