● 722 VITAMIN B_{12}-RESPONSIVE MEGALOBLASTIC ANEMIA AND HOMOCYSTINURIA: DESCRIPTION OF TWO COMPLEMENTATION CLASSES, cblE AND cblG. D.S. Rosenblatt and D. Watkins. McGill University, MRC Genetics Group, Centre for Human Genetics and Depts. of Medicine, Pediatrics, and Biology, Montreal, Quebec.

The cblE disorder is characterized by megaloblastic anemia of infancy associated with homocystinuria but no methylmalonic aciduria (N Eng J Med 310:686, 1984). Untreated, this disorder results in developmental delay. In cultured fibroblasts from the original proband, there was decreased synthesis of methyl-B_{12} and decreased methionine biosynthesis. Activity of the methyl-B_{12}-dependent enzyme methionine synthase in cell extracts was decreased only when assayed in the presence of suboptimal reducing conditions (J Clin Invest 74:2149, 1984). We have studied cultured fibroblasts from several additional patients with similar clinical findings. Methyl-B_{12} synthesis and methionine biosynthesis were decreased. Methionine synthase activity in fibroblast extracts was decreased even under optimal reducing conditions. Complementation studies were carried out using polyethylene glycol-induced fusion of cells from these various patients. Methionine biosynthesis was increased 2.21-fold, 2.45-fold and 1.41-fold respectively in fused mixed cultures of cells from the original proband with cblE with cells from 3 of the subsequently diagnosed patients. No complementation occurred between cells from these 3 patients. These results identify two distinct complementation classes and suggest that mutations at more than one locus can give rise to this clinical condition. We have called the new complementation class "cblG".

723 CHROMOSOMAL ASSIGNMENT OF THE HUMAN GENE FOR THE TRIFUNCTIONAL FOLATE-DEPENDENT ENZYME: EVIDENCE FOR MULTIPLE LOCI. Rima Rozen[1,2], Dean W. Hum[3], David Barton[4], Uta Francke[4] and Robert E. MacKenzie[3] (Spon. by Charles R. Scriver). McGill University, Depts. of Pediatrics[1], Biology[2] and Biochemistry[3], Montreal, Quebec and Yale University, Dept. of Human Genetics[4], New Haven, Connecticut

The eucaryotic trifunctional protein (5,10-methylenetetrahydrofolate dehydrogenase -5,10-methenyltetrahydrofolate cyclohydrolase-10-formyltetrahydrofolate synthetase) catalyzes 3 consecutive steps in the interconversion of tetrahydrofolate derivatives; tetrahydrofolates are the one-carbon units required for the synthesis of thymidylate, purines and methionine. We have recently described the isolation of a human liver cDNA of 250 bp, coding for this protein, from a λgt11 expression library (Amer. J. Hum. Genet. 39: A204, 1986).

In this communication, we report the chromosomal mapping of the gene in somatic cell hybrids. Southern blot analysis of BamHI-digested DNA reveals 2 prominent bands in humans (~ 19 and 14 kb) and one major band in Chinese hamsters (~5 kb). In Chinese hamster X human hybrids, the 19 kb band is localized to human chromosome 14 (q_{21}-q_{ter}) and the 14 kb band, to the X chromosome. The assignment of the gene to separate loci suggests the presence of 2 homologous genes, or, more likely, the existence of a pseudogene. Ongoing studies with longer cDNA probes will enable us to determine the exact number of homologous genes in this family.

● 724 HETEROZYGOTE DETECTION IN CYSTINOSIS USING POLYMORPHONUCLEAR LEUKOCYTES. Lori A. Smolin, Karen F. Clark and Jerry A. Schneider, University of California, San Diego, Department of Pediatrics, La Jolla, California.

Heterozygotes for the autosomal recessive disease cystinosis are currently detected by measuring the cystine content of mixed leukocyte preparations. This method offers about 90% accuracy. The following study was designed to determine if measuring the cystine content of purified preparations of polymorphonuclear leukocytes (PMNs) would improve the accuracy of heterozygote detection. Subjects included 29 obligate heterozygotes for nephropathic cystinosis and 18 individuals presumed to be normal. Mixed leukozytes were prepared from 10 ml of blood by dextran sedimentation; PMNs were prepared by centrifugation of 4.5 ml of blood on a discontinuous gradient of Ficoll-Hypaque. The cystine content of both leukocyte preparations was determined by a specific binding assay. All values are expressed as nmol 1/2 cys/mg protein and are shown in the table below. Using mixed leukocyte preparations, 3 heterozygotes overlapped the normal range reconfirming a detection accuracy of 90%. Using PMNs no heterozygote values fell within the normal range. Measuring the cystine content of PMNs appears to provide a simple screening assay which improves the accuracy of heterozygote detection for cystinosis.

	Mixed Leukocytes		PMNs	
	range	mean±SD	range	mean±SD
Normals	0.03–0.11	0.06±0.02	0.06–0.22	0.14±0.05
Heterozygotes	0.07–1.10	0.28±1.10	0.30–1.45	0.70±0.33

● 725 PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS (CF) USING LINKED DNA MARKERS AND MICROVILLAR INTESTINAL ENZYME ANALYSIS. J. Edward Spence, Gregory J. Buffone, Susan D. Fernbach, Martha R. Curry, William E. O'Brien and Arthur L. Beaudet, Howard Hughes Med. Inst. and Baylor Col. Med., Inst. for Molecular Genetics and Depts. of Pediatrics and Pathology, Houston.

We are performing prenatal diagnosis of CF in families with 1 in 4 risk using linked DNA markers and amniotic fluid microvillar intestinal enzyme (MIE) analysis. The probes and RFLPs used for DNA analysis are as follows: metH with MspI and TaqI; metD with TaqI and BanI; D7S8 with MspI and TaqI. Using these probes in over 100 couples at 1 in 4 risk, 77% were fully informative. We have studied 53 pregnancies with 1 in 4 risk using DNA alone in 11, DNA and MIE in 26, and MIE alone in 16. DNA samples were obtained by chorionic villus sampling in 13 cases and by amniocentesis in 24 cases. For the 26 cases studied by DNA and MIE, results of both tests were conclusive and in agreement for 20 cases, and one test was diagnostic in 4 of the remaining cases. In only 2 of the 53 cases studied, no adequate diagnosis was achieved, because the fetus was at 50% risk by DNA data and MIE analysis was inconclusive. For 13 pregnancies predicted to be affected, 7 were terminated, 5 were born affected and 1 is awaiting outcome. For 37 pregnancies predicted to be unaffected, 11 were born unaffected and 26 are awaiting outcome. Assuming a recombination fraction of 0.01 between CF and the DNA loci, molecular prenatal diagnosis for affected or unaffected status would be 96%, 98% or >99% accurate if the fetus is predicted to be affected, carrier or noncarrier respectively. Use of flanking DNA markers or combination with MIE analysis can increase accuracy to >99%.

● 726 PIGMENTARY ABNORMALITIES AND CHROMOSOMAL AND GENETIC MOSAICISM AND CHIMERISM Ioan T. Thomas, Eduardo S. Cantu, and Jaime L. Frias, Division of Genetics, Dept of Pediatrics, University of Florida, Box J-296, JHMHC, Gainesville, Florida. Charlotte Lafer, Dept of Peds, Univ Hospital, Jacksonville,FL. David B. Flannery, Div. Med. Gen., Dept of Peds, Med. Coll. of Georgia, Augusta, GA. John M. Graham Jr., Dept. Mat. and Child Health, Dartmouth Univ. Med. School, Hanover, New Hampshire.

We have studied nine patients who presented with pigmentary anomalies of the skin in association with other congenital malformations; in eight, we demonstrated chromosomal mosaicism in lymphocytes and/or skin fibroblasts. A review of the literature revealed 25 similar examples of an association between pigmentary anomalies and chromosomal mosaicism or chimerism. The pigmentation varied but usually followed a pattern known as Blaschko lines. Our findings indicate a relationship between aberrant skin pigmentation and chromosomal mosaicism, and suggest a developmental explanation for the pigmentary patterns seen in chromosomal or genetic mosaicism and in chimerism. Further, they emphasize the need for extensive cytogenetic investigation of patients who present with pigmentary abnormalities and associated malformations.

727 DOMINANTLY INHERITED OSTEOGENESIS IMPERFECTA (OI TYPES I AND IV) IS GENETICALLY LINKED TO THE COL1A1 AND COL1A2 GENES OF TYPE I COLLAGEN. Petros Tsipouras and Robin C. Schwartz (Spon. by N. Sissman). University of Connecticut Health Science Center, Farmington, CT.

Osteogenesis imperfecta (OI) is a genetically heterogeneous group of connective tissue disorders. Mutations in Type I collagen have been shown in a number of OI variants. Using restriction fragment length polymorphisms (RFLPs) associated with the two structural genes of Type I collagen we studied the segregation of these markers in families with autosomal dominant OI. The RFLPs have been previously reported (Tsipouras et al, Am.J. Hum. Genet. 1984: 36:1172-9, Sykes et al, Lancet 1986:2:69-72). In four families with OI type IV we observed concordant segregation to the COL1A2 RFLPs and discordant to the COL1A1. In one family with OI type I we observed concordance to the COL1A1 and discordance to the COL1A2. Finally in one family with OI type I we observed discordance to both the COL1A1 and COL1A2. Our results suggest that: a) autosomal dominant OI is genetically heterogeneous and b) a locus other than the COL1A1 and COL1A2 may be involved in the etiology of OI.