● 710 MOLECULAR CLONING OF cDNA ENCODING RAT ISOVALERYL-CoA DEHYDROGENASE (IVD). Yoichi Matsubara, Jan P. Kraus, Robin Glassberg, Michinori Ito, Yasuyuki Ikeda, John Mole*, Leon E. Rosenberg, and Kay Tanaka. Yale Univ. Sch. of Medicine, New Haven, CT and *Univ. of Massachusetts, Worcester, MA (Spon. by Leon E. Rosenberg).

IVD is a mitochondrial flavoprotein, a tetramer of 43 kDa subunits, which catalyzes the third reaction in leucine metabolism. Hereditary deficiency of IVD causes isovaleric acidemia, one of the more common inherited organic acidemias, characterized by recurrent episodes of vomiting, ketoacidosis, lethargy and sweaty feet odor. Extensive molecular heterogeneity, including at least five distinct variant alleles, has been shown on the basis of the presence or absence of immunoprecipitable variant IVDs and their sizes (PNAS 82:7081, 1985). As a first step in the study of the molecular basis of isovaleric acidemia, we have isolated a cDNA clone encoding rat IVD. First, we highly purified the mRNA encoding IVD from rat liver by polysome immunopurification using polyclonal, monospecific antibody. A cDNA library, enriched for IVD, was prepared using the purified mRNA, and screened by colonly hybridization using two oligonucleotides (17-mer and 15-mer) corresponding to the portions of the NH_2-terminal amino acid sequence. Numerous positive cDNA clones were isolated. One of them contained a 54 bp portion which perfectly matched an 18 amino acid peptide from the amino terminus of pure rat IVD, thereby positively identifying the clone as that of rat IVD. This cDNA extends 100 bp towards the 5' end, indicating that it contains the entire sequence for the leader peptide (2 kDa). Screening of human cDNA libraries for IVD cDNA is in progress.

711 MOLECULAR CLONING AND NUCLEOTIDE SEQUENCE OF cDNA ENCODING RAT MEDIUM CHAIN ACYL-CoA DEHYDROGENASE (MCAD). Yoichi Matsubara, Jan P. Kraus, Hisashi Ozasa, Gaetano Finocchiaro, Robin Glassberg, Yasuyuki Ikeda, John Mole*, Leon E. Rosenberg, and Kay Tanaka. Yale Univ. School of Medicine, New Haven, CT and *Univ. of Mass., Worcester, MA (Spon. by Leon E. Rosenberg).

MCAD is a mitochondrial flavoprotein, a tetramer of 45 kDa subunits, which catalyzes the first reaction in the β-oxidation of medium chain fatty acids. Hereditary MCAD deficiency causes recurrent episodes of non-ketotic hypoglycemic coma, accompanied by medium chain dicarboxylic aciduria. We recently reported the isolation of partial cDNAs encoding rat and human MCAD, containing 50 and 80% of the coding region, respectively. The gene for human MCAD was assigned to chromosome 1, p31 (PNAS 83:6543, 1986). We report here the isolation and nucleotide sequence of a cDNA clone that contains the entire coding region of rat MCAD. mRNA encoding MCAD was purified from rat liver by polysome immunopurification using monospecific antibody, and a cDNA library enriched from MCAD was constructed. The library was screened by colony hybridization using a previously isolated rat MCAD cDNA clone. Numerous MCAD clones were isolated. In vitro expression of the longest clone (1860 bp) yielded a polypeptide that is indistinguishable in size from rat precursor MCAD containing the leader sequence. This peptide was immunoprecipitable with anti-MCAD antibody. The identity of the clone was further confirmed by comparison of the entire nucleotide sequence with amino acid sequences of the amino terminal and six internal peptides of rat MCAD. The leader peptide contains 25 amino acids, and is rich in basic amino acids (4 Arg, 1 Lys).

● 712 L-PIPECOLIC ACID IS OXIDIZED TO α-AMINOADIPIC ACID IN THE PEROXISOME OF MAN AND MONKEY. Stephanie J. Mihalik and William J. Rhead. University of Iowa, College of Medicine, Dept. of Peds., Iowa City, IA.

L-Pipecolic acid (PIP) accumulation is found in Zellweger syndrome (ZS) and other diseases where the structure or function of peroxisomes is defective. Since there is no primate model for PIP metabolism, we compared the pathway in the cynomologus monkey to that in man. Monkey kidney cortices and livers were homogenized and crudely fractionated by differential centrifugation. After incubation with L-[2,3,4,5,6-^{3}H]PIP, reaction products were separated by thin layer chromatography and on the amino acid analyser. The only radioactive product comigrated with authentic α-aminoadipic acid (AAA) in all systems employed. PIP oxidation was highest in the light mitochondrial (L) fraction, as was activity of catalase, a peroxisomal marker enzyme. After further separation on a Percoll gradient, PIP oxidation again paralleled catalase activity in both liver and kidney. In purified monkey peroxisomes, the activity was membrane associated and could be released by 100 mM KCl. Structural analogs of PIP, such as L-proline, nipecotic acid, piperidine, and cis-2,4-dicarboxypiperidine, were not inhibitory. In fresh human liver fractionated on a Percoll gradient, PIP oxidation also paralleled catalase activity. Activity was only 6% of control in liver homogenate from a ZS patient (courtesy of Hugo and Ann Moser, Baltimore). The association of the activity with the membrane may explain why PIP metabolism is impaired in disorders where peroxisomal membrane assembly or biogenesis is defective.

● 713 FIRST TRIMESTER MATERNAL SERUM ALPHA-FETOPROTEIN (MSAFP) SCREENING FOR CHROMOSOME DEFECTS. Aubrey Milunsky, Jack R. Wands, Bruno Brambati. Boston Univ.Sch. Med., Ctr. for Human Genetics, Mass. Gen. Hosp., Div. Gastroenterol., Dept. Med., Boston, & Dept. Obstet. & Gynecol., Univ. of Milan, Italy.

Second trimester MSAFP screening for low values is proving valuable in the detection of fetal chromosome defects in women < 35 yrs. of age. Detection prior to 16 wks. would be desirable. To determine whether low MSAFP values also occur in the first trimester in association with a fetal chromosomal abnormality, we used a "simultaneous-sandwich" radioimmunoassay with polystyrene beads coated with anti-AFP monoclonal antibodies, using solid phase support. The coefficient of variation was < 5% for this assay which is about 10 x more sensitive than conventional RIAs.

Blood was sampled in 359 cases just before CVS (chorionic villus sampling) in Milan. Fetal age was usually assessed by 2 ultrasound studies ± 1 wk. apart, and chromosomes of the villi analysed. Sera were sent blind & only after AFP assay results were sent to Milan were karyotypes released. MSAFP ≤ 0.6 MOM occurred in 5/17 (45.5%) significant fetal chromosome defects (trisomy 21,18[2],13 & unbal. 13/14 translocation) while 2 (18.2%) were < 0.5 MOM. 11/17 (64.7%) affected fetuses had MSAFP < median. 1/5 trisomy 21 fetuses had MSAFP < 0.5 MOM.

About 75% of trisomy 21 offspring are delivered by women < 35 yrs. of age. These results suggest that first trimester MSAFP screening could identify a significant number of these pregnancies early enough for CVS.. Further study is necessary to determine the optimum week for efficient screening.

● 714 ORNITHINE AMINOTRANSFERASE (OAT): EVIDENCE FOR A DISPERSED GENE FAMILY WITH MEMBER(S) LOCALIZED TO Xp11.1 - Xp21.1. Grant Mitchell, David Valle, Maureen Suchanek, Gary Steel, Lawrence Brody, James Looney & Hunt Willard, Johns Hopkins Univ School of Med, Dept of Peds, Balto and Dept of Med Genetics Univ of Toronto, Ontario.

OAT is a homotetrameric, mitochondrial matrix enzyme deficient in the autosomal recessive retinal degeneration, gyrate atrophy of the choroid and retina. We have cloned and sequenced a near full length human OAT cDNA. We used this to probe genomic DNA digested with various restriction enzymes and found up to 15 OAT-hybridizing fragments (OAThf). They map to 2 locations: 1/3 are on chromosome 10 (10-OAThf) and represent the OAT structural gene; 2/3 are on Xp11.1-21.1 (X-OAThf). Examination of primate DNAs show that this division of X- and autosomal-OAThf has existed for at least 25 million yrs. The X-OAThf do not hybridize to a near full-length cDNA for ornithine transcarbamylase, another ornithine-metabolizing enzyme mapping to Xp21.1. We isolated OAT-hybridizing genomic clones from chromosomes 10 and X. Preliminary sequence analysis of one X clone shows discrete blocks of high nucleotide homology (92%) to OAT cDNA with in-frame stop codons and typical intron-exon junctions indicating that it is a non-processed pseudogene of either OAT or another OAT-related gene. Comparison of the sequences of clones from 10 and X shows similar but not identical positioning of intron-exon junctions. These results suggest that the OAT structural gene on 10 is a member of a small dispersed gene family with a non-processed pseudogene and probably other members all localized to Xp11.1 to Xp21.1.

● 715 EVALUATION OF RETROVIRAL MEDIATED GENE TRANSFER BY IN SITU HYBRIDIZATION Robert C. Moen, R. Michael Blaese, and W. French Anderson NIH, Bethesda, MD

Genetically engineered retroviral vectors can be used to transfer genes into eucaryotic cells. The application of these vectors for human gene therapy is being evaluated in vitro and in animal models. The time course of vector mediated gene transfer into the human erythroleukemia cell line, K562, was followed by in situ hybridization (ISH) to determine if this technique could be used to follow retroviral mediated gene transfer. The initial binding of vector to K562 cells was readily detected. Little vector RNA was detected during particle uptake, conversion of the vector RNA to dsDNA, and integration of the dsDNA into the host genome. However, by 18h after vector binding cytoplasmic RNA from the transferred gene was readily detected with steady state levels reached by 48h, demonstrating the ability of ISH to follow vector mediated gene transfer. In an autologous monkey bone marrow transplant model attempts to transfer a human adenosine deaminase (hADA) gene has resulted in hADA activity 0.5% that of endogenous monkey ADA. To determine if this represents a low level of activity in a large number of cells or the converse, ISH was used to show that 6 of 673 peripheral blood mononuclear cells contained retroviral RNA, implying an appropriate level of hADA gene expression in those cells infected by the vector. Similar percentages of gene transfer are seen in in vitro studies with primate and human bone marrow colony forming assays. The ability of ISH to detect expression of transferred genes in single cells provides a powerful technique for the refinement of methods for human gene therapy.