platform presentations in **biochemical** genetics

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Five years' experience of cholesterol treatment for the Smith-Lemli-Opitz Syndrome (SLOS): What have we learned, where are we going? ER Elias[1], M Irons[2], GS Tint[3] and S Salen[4]. Children's Hospital, Boston, MA[1,2] and VA Medical Center, E Orange, NJ [3,4]

Since January of 1994, following the discovery of the defect in cholesterol metabolism now known to cause SLOS, we have been treating SLOS patients with a concentrated suspension of cholesterol in soybean oil. Eighteen patients with SLOS, ranging in age from birth to mid teens, and ranging in pretreatment cholesterol levels from 8-90 mg/dl have been followed by us every 1-3 months for up to five years while on treatment.

We will present data to show benefits of treatment in this cohort of patients. No adverse reactions to therapy have been noted. Benefits of therapy include improved growth and nutritional status, better tolerance of infection, and improvement in behavioral disturbances. Adrenal function and neutrophil function have been investigated, and both are normal. Ongoing studies are documenting neuropsychological course, CNS myelinization on MRI, and hearing status with ABR.

We have discovered problems in our patients which were not previously known to occur in SLOS. These include photosensitivity, which we are now studying both in vitro in skin fibroblasts, and in vivo using timed exposure to UV light. We have also documented abnormal red cell shape (acanthocytosis), and the development of hypersplenism with thrombocytopenia in some patients. We are now accumulating experience in the management of profoundly ill neonates using fresh frozen plasma, and in prenatal diagnosis, by assessing low maternal serum estriol levels. Future plans include studies of cholesterol absorption, and the use of MR spectroscopy to assess CNS cholesterol metabolism. Our close follow-up of this patient cohort has allowed us to appreciate the complexities of issues seen in children with severe cholesterol deficiency, and to develop a greater understanding of the natural course of SLOS.

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Cholesterol supplementation enhances growth of phallus in Smith-Lemli-Opitz syndrome. M.B. Irons [1], T. L. Stewart [2], A. Sadeghi-Nehad [2]. [1]Children's Hospital, Harvard Medical School, Boston, MA, [2] Floating Hospital for Children at New England Medical Center, Tufts University School of Medicine, Boston, MA.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive inborn error of cholesterol biosynthesis due to a deficiency of the last enzyme of the cholesterol synthetic pathway, 7-dehydrocholesterol reductase. Affected patients have low concentrations of cholesterol and elevation of the cholesterol precursor, 7-dehydrocholesterol (7-DHC). Multiple congenital anomalies, dysmorphic facies, and growth and developmental retardation are common. Severely affected males have ambiguous genitalia. We believe that the genital anomalies seen in affected males are due to deficiency of androgens, but this has never been proven.

The infant was diagnosed prenatally with SLOS by sterol analysis of amniotic fluid at 30 weeks gestation. Physical examination on Day 1 of life revealed many of the physical features of SLOS. Biochemical studies confirmed the prenatal diagnosis. The penis measured 1 X 0.7 cm, and was hooded and ventrally bound. He had a third-degree hypospadias and a bifid scrotum. There was hyperpigmentation of the nipples and scrotum.

Treatment with exogenous cholesterol (150 mg/kg/day) resulted in an increase in the size of the phallus to 2.3 X 1.2 cm in six weeks. Biochemical studies before and after therapy revealed the following: cholesterol of 21 8 and 26 mg/dl; 7-DHC of 7.25 and 12 mg/dl; ACTH of 150 and 77 pg/ml; cortisol of 9.6 and 14 mcg/dl; testosterone of 48 and 153 ng/dl; and dihydrotestosterone of less than 2 and 38 ng/dl.

We conclude that in males with SLOS treatment with cholesterol improves biosynthesis of cortisol and androgens, and enhances phallic growth.

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Autism associated with elevated glutamine and glycine levels and clinical response to dextromethorphan. R. Hamid, S. McGrew and J.A. Phillips III. Dept. Peds, Vanderbilit Univ. Schl of Medicine, Nashville, TN.

Autism is a pervasive developmental disorder characterized by social relating and communicating impairments and restricted, repetitive or stereotypical behavior with onset by 3 years of age. A genetic etiology is suggested by developmental anomalies; increased risks for sibs and concordance for monozygotic twins. In screening a series of consecutive autistic probands, we detected 2/60 who had elevated plasma levels of glutamine (Glu) and glycine (Gly). The first was diagnosed as having autism at 2 $^{10}/_{12}$. His Glu (mean 884-normal 370-682) and Gly (mean 379-normal 120-315) levels were consistently elevated on all 5 studies done between 4 $^{11}/_{12}$ and 6 years of age. His Phe (mean 86-normal 39-78) and Ser (mean 185-normal 31-131) levels were usually elevated. Urine Glu, Gly, Phe and Ser levels were 1410 (165-510), 5663 (569-1395), 134 (27-51) and 1163 (148-360). His CSF Glu was 708 (normal 356-680) while his Gly, Phe and Ser levels were normal. His electrolytes, anion gap and plasma ammonia levels were all well within the normal range and his urine organic acid profile was normal. To competitively block his elevated Glu and Gly levels he was empirically treated with dextromethorphan (DM) at 5 mgm/kg/day (Delsym) divided BID. His special education and classroom teachers and both speech and occupational therapists (blinded to treatment) noted significant improvement in his expressive and receptive language skills, attention span and focus; motor planning and socialization with peers. After withdrawal of DM regression in all improved areas was noted by all of these treatment blinded observers. He continues to respond to resumed, long term DM treatment. Our data suggest some autistic children have consistent elevations of Glu and Gly and they may respond clinically to DM. Further studies are needed to determine the cause of these biochemical findings and the responce of other subjects to DM or other competitive inhibitors.

4

Diagnosis of Gaucher disease by a flow cytometric assay. H. J. Kim,[1,2] M. J. Ha,[1] H. W. Kang,[1] M. S. Yang,[3] H. S. Kim,[3] and H. C. Kim[3]. [2]Genetics Clinic, [1]Laboratory Medical Genetics, [3]Department of Hemato-oncology, Ajou University College of Medicine, Suwon, Korea

Gaucher disease (GD) is an inherited autosomal recessive disorder, caused by a deficient lysosomal β-glucocerebrosidase (GC) resulting in the acumulation of the glycoslyceramide in the lysosomes of cells of the reticuloendothelial system. Measurement of GC activity during the emzyme replacement therapy and following allogeneic bone marrow transplantation or gene therapy would be useful. GC activity is commonly measured using radiolabeled glucocerebroside in cell lysates. This method of *in vitro* assay does not allow one to measure GC activity on a single cell basis. The fluorescence-activated cell sorter (FACS) has been used for the measurement of GC activity in single cells. To determine the presence of enzyme dificiency for the diagnosis of GD and monitor the efficacy of enzyme replacement therapy, we examed a flow cytometric assay for GC using 5'-pentafluorobenzoyllaminofluorescein-di-β-D-glucoside (PFBFDGlu) which has been reported to be a substrate for GC. The study group consisted of 11 normal individuals, 14 obligatory carriers (parents of patients), and 11 known Gaucher patients (8 of type 1, 1 of type 2, and 2 of type 3). The peripheral blood mononuclear cells obtained by ficoll density centrifugation were washed three times in RPMI 1640 containing 10 % FBS and immediately processed for substrate loading. To determine the optimal assay condition, 5 x 10^5 cells were mixed with an equal volume of 1 mM PFBFDGlu (0.5 mM final) and incubated at 37℃ for 0 to 60 min or cells were incubated for 30 min at 0.5, 1, and 2 mM PFBFDGlu. To terminate substrate loading, 0.5 ml of ice-cold RPMI 1640 containg 4 % FBS was added to the cell suspension. FACS analysis was done on a FACSTAR Plus. FACS analysis revealed that PFBFDGlu loaded normal or carrier cells show brighter median fluorescence (MF) levels than patients' cells at all incubation time points, the fluorescence distributions show minimal overlap with a 30-min incubation. With longer incubation times, however, fluorescence histograms of cells became increasingly broader, and the determination of MF was difficult. At our optimal assay condition (0.1 mM PFBFDGlu, 30 min incubation), the fact that fluorescence distributions of all the tested cells from patients (6.237 ± 1.866, n=27) did not overlap with those from carriers (13.704 ± 4.232, n=27) or from normal individuals (11.857 ± 1.351, n=14) indicating this simple and fast flow cytometric GC assay will be useful for diagnosis of GD. Furthermore, in our study, some population of cells which had increased GC activity can be detectable in cells from Gaucher patient after treatment with Cerezyme® indicating this method can be useful for monitoring effective dosage and frequency of enzyme replacement therary from each patients, as well as the efficacy of gene therapy for Gaucher patient in future.