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Report on the scientific sessions by

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The scientific sessions held at the **United Leukodystrophy Foundation Scientific Symposium, 2006**, entitled “The Expanding Spectrum of White Matter Disorders: Approaches for Diagnosis and Treatment” enabled a fruitful exchange between clinicians and scientists.

New research probing disease mechanisms were brought forth by young investigators. While newborn screening focused on defining the parameters of known diseases, novel diagnostic methods were presented that expanded our knowledge of the spectrum of white matter disorders. The success of interventions in the human as well as in the animal model was subject of vigorous discussion. The coming years will likely further change our perspective upon leukodystrophies. The exchange of observation between bench and bedside will prepare us and help us implement the scientific progress to the benefit of our patients.

Robert Jan Sanders, MSc. from the Amsterdam Medical Center, Netherlands, gave a talk on omega-oxidation of very long chain fatty acids (VLCFA) and its implication for X-linked adrenoleukodystrophy (X-ALD). In X-ALD there are mutations in the ABCD1 gene which encodes a protein responsible for transport of VLCFA into the peroxisome. In the peroxisome VLCFA get broken down via beta oxidation. However, there is an alternative breakdown route for VLCFA via the omega-oxidation which takes place in a separate compartment of the cell. Here VLCFA are converted to dicarboxylic acid and are subsequently degraded. This pathway accounts for 5-10% of total fatty acid oxidation in the liver and is catalyzed by a group of enzymes, called P450 enzymes. Analyses of fibroblasts of patients with X-ALD show the ability of VLCFA to undergo omega-oxidation and to metabolize long chain dicarboxylic acids. In future stimulation of these enzymes may reveal options for therapeutic interventions in X-ALD. This was exciting news, since it revealed that the protein defective in ALD may not be an absolute necessity for VLCFA degradation.

Dr. Yoishi Kondo from Waisman Center, Madison, spoke about the roles of microglia and macrophages in Krabbe’s disease. In Krabbe’s Disease the deficient enzyme is galactosyl ceramidase (GALC), which is needed to break down galactosylceramide. GALC deficiency causes accumulation of psychosine, which leads to cell death of oligodendrocyte and neuron with subsequent myelin arrest. Galactosylceramide accumulates in globoid cells and produces a macrophage response. The exact function of the phagocytic cell response is unclear. To explore this further, a mutated macrophage deficient mouse was crossed with the twitcher mouse, an animal model of Krabbe’s disease. This double mutant mouse not only had a drastic decrease in macrophages but was also deficient in GALC. Unfortunately the macrophage deficiency did not protect against the demyelinating pathology. The double mutant developed tremor earlier and became paralyzed and moribund 5 days earlier than the twitcher mouse. Hence it appears that microglia and macrophages are likely protective in Krabbe’s disease. This may also be of importance in considering enzyme delivery to the central nervous system, since macrophages and microglia may be used as a vessel to deliver GALC to the brain. Currently mice survival

after bone marrow transplantation is only 100 days. Improved delivery systems and GALC overexpression may extend this or allow starting therapy later. This has immediate implications for the human disease where enhancing enzyme delivery to the brain continues to be paramount.

Dr. Marc Engelen from the Amsterdam Medical Center gave a talk on cholesterol deprivation in X-linked adrenoleukodystrophy (X-ALD). In fibroblasts of X-ALD patients lovastatin, a lipid lowering agent, is known to decrease very long chain fatty acids (VLCFA) and thereby reverse the biochemical defect in X-ALD. This has been thought to occur through increased degradation via beta-oxidation as well as expression of the ALD-related gene, a gene that shares properties with the defective gene in X-ALD. Dr. Engelen explored whether the reduction in VLCFA could occur through an alternative pathway. He observed that in culture medium cholesterol deprivation induces a shift from saturated to monounsaturated VLCFA. This can be reversed by adding cholesterol. Hence the observed lowering of VLCFA by lovastatin may be caused by a shift to monounsaturated VLCFA and not increased degradation. This remains to be explored in lovastatin treated fibroblasts. This is potentially important, since it points to the importance of a low fat diet and may explain the lack of response to lovastatin in some patients.

Dr. Bams-Mengerink from Amsterdam Medical Center presented on batyl alcohol supplementation in rhizomelic chondrodysplasia punctata 1 (RCDP1). RCDP1 is a peroxisomal disorder characterized by facial dysmorphism, congenital cataracts, symmetric shortening of the upper limbs, calcific stippling and severe mental retardation. However, there is a wide spectrum of the disease. At the metabolic level, RCDP patients have defects in just two peroxisomal metabolic functions: plasmalogen biosynthesis and branched chain fatty acid oxidation. Plasmalogen biosynthesis occurs in the peroxisome and endoplasmic reticulum. The addition of alkyl glycerol may bypass the peroxisomal step and normalize plasmalogen levels in RCDP patients. Shark liver oil is rich in alkyl alcohol and its use showed a mild benefit on red blood cells but unfortunately phytanic acid accumulated more rapidly than before. As an alternative batyl alcohol is currently being studied in a clinical trial in the Netherlands. So far 6 patients with RCDP1 have been tested and tolerated the treatment well. Batyl alcohol increases plasmalogen levels in their red blood cells but it is unclear whether this leads to clinical improvement.

Dr. Walter Hubbard from Johns Hopkins, Baltimore, presented preliminary data on newborn screening in X-ALD. With a number of therapies being available in this disorder, newborn screening has recently been advocated. It is known that saturated very long chain fatty acids accumulate in the plasma of X-ALD but it has been difficult to detect this on dried blood spots obtained by newborn screening. Dr. Hubbard introduced a method that simultaneously performs liquid chromatography tandem to different mass spectrometry techniques and can thereby demonstrate a fraction of phospholipids containing an excess in very long chain fatty acids. In 25 patients and 19 controls there was no overlap between affected and normal subjects. A larger scale of testing is still needed to verify these preliminary results and automation for high throughput screening still remains a challenge. However, it is promising that this assay can be done on the small dried blood spot and with the same instrument that is used for newborn screening. Early screening will enable us to monitor the brain and adrenal gland in X-ALD. Also Lorenzo's oil can be started earlier and thereby help reduce the risk of cerebral demyelination.

Dr. Kenneth Pass from University of Albany, New York, and **Dr. Ron Scott** from University of Washington, Seattle, spoke about newborn screening for lysosomal storage diseases. Tandem mass spectrometers can measure amino acids and metabolites on dried blood spots. On newborn screening these measurements help us identify treatable diseases. With the advances in treatment availability newborn screening has now been developed for a number of lysosomal storage

disorders, such as Fabry, Gaucher, Hurler, Krabbe, Niemann-Pick A/B and Pompe diseases. These diseases were selected because treatments are now available or expected to emerge shortly.

Screening for deficiencies is far more difficult than detecting elevations of a substance and poses a major barrier when attempting to distinguish affected individuals from carriers. However, the developed methodology can reliably measure low activity in control samples. In the case of Krabbe's disease, all patient samples showed less than 50% of the mean enzyme activity in controls. The screening laboratories continue to evaluate cutoffs and adjust their parameters based on results of confirmatory tests but problems with invalid specimen handling and false positive rates are challenging. The newborn screening assay for Krabbe disease is currently in place at the Wadsworth Center in New York State for the analysis of approximately 1000 dried blood spots per day. So far it has detected 6 affected Krabbe patients and 14 heterozygotes. As with the other lysosomal storage disorders the question remains how to implement a clinical infrastructure to accommodate all the new patients identified by newborn screening.

Dr. Nancy Braverman from the Kennedy Krieger Institute, Baltimore, talked about new therapeutic approaches for peroxisome biogenesis disorders. These disorders are due to defects in peroxisome assembly genes, referred to as PEX genes. Twelve PEX genes are required for normal organelle assembly. They regulate targeting and importation of peroxisomal integral membrane and matrix proteins. Understanding these disease mechanisms helps us identify new means of intervention. Dr. Braverman reported on the use of screening chemical libraries for the rescue of peroxisome assembly defects, in particular importation defects. She further reported on the influence of temperature upon protein assembly and presented data on improved folding of the PEX6 protein at lower temperatures. Again this insight into molecular mechanisms may guide therapeutic approaches in the future.

Dr. Ed Kaye from Genzyme Corporation, Cambridge, spoke about gene therapy for Niemann Pick Type A (NP-A). NP-A is an inherited disorder that causes lipid storage and damage to liver and brain and gene delivery in the animal model of NP-A has been an intense research focus of Genzyme. The corrected gene packaged into a viral vector can be directly injected into cortex, basal ganglia, cerebellum and brainstem. This was able to reverse the abnormal lipid storage and pathology and preserve brain function. The combination of systemic injections and brain injection has recently been explored. While the NP-A knockout mouse dies around 37 weeks, the animals with systemic injection alone and brain injection alone lived longer and with combination therapy motor and cognitive function improved even more. Levels of human ASA in brain increased the most with the combination therapy, likely due to an altered immune response in the brain. Hence it appears that visceral and brain treatments are synergistic and result in significant improvement in survival compared to systemic injection alone and brain injection alone. This observation may have wider relevance for other neurodegenerative disorders.

Dr. Ulrich Matzner from the Rheinische Friedrich-Wilhelms-Universitaet Germany discussed the therapeutic efficacy and side effects of enzyme therapy for the metachromatic leukodystrophy (MLD) mouse model. MLD is a lysosomal storage disorder caused by deficiency of Arylsulfatase A (ASA). Deficiency of ASA causes accumulation of a sphingolipid, sulfatide which accumulates in oligodendrocytes and causes demyelination. In the mouse model of MLD, enzyme replacement therapy (ERT) can reduce 70% of the excess sulfatide with repeated treatments over a four-week period (large doses of 20mg/kg per week injected into the tail vein). Yet side effects remain a major limitation and include anaphylactic reactions related to antibodies to human ASA. Further, treatment resistance occurs in some mice. Since both these

problems are related to antibody production, the mouse model was examined for inhibitory antibodies. In the mouse no antibodies to ASA or saposin B were detected but in a cell culture model redistribution of ASA was observed. It is currently thought that antibodies affect targeting of the human ASA and that anti human ASA antibodies redirect ASA and lead to internalization into a separate compartment and subsequent rapid degradation. Attempts at improving the mouse model of MLD have focused on creating immune tolerant mice as well as mice with a pathology more closely resembling the human. The latter has been accomplished by increasing synthesis of galactosylceramide and sulfatide on the background of the ASA KO mouse. These mice have a more severe phenotype resembling that of MLD.

Dr. Larry Charnas from the University of Minnesota reported on treatment in advanced cerebral X-linked adrenoleukodystrophy (X-ALD). In early X-ALD bone marrow transplant has been successful and offers reasonable benefit. However, treatment options for more advanced boys are scarce and desperately needed. In autopsy specimen of cerebral ALD there is compelling evidence for oxidative stress. Nitrogen species and products of lipid peroxidation may contribute to cell damage and cause rapid cell lysis. Given this observation N-acetylcysteine (NAC), an agent that counters oxidative stress, was tested at the University of Minnesota. As part of the preregimen for bone marrow transplantation it was given to six X-ALD patients with advanced brain disease. They are now between 5 and 8 months post transplant. Vision was preserved in one patient who prior to transplant already showed progressive deterioration of vision. Despite marked atrophy post transplant the white matter lesions did not progress in these patients. Unfortunately NAC in these patients was given in varying combination regimens making definite conclusions difficult. However, the findings suggest that less neurotoxic myeloablative therapy can be recommended. Further it appears beneficial to avoid agents that generate free radicals as part of their mechanism of action.

Dr. Marjo van der Knaap from the Free University Medical Center, Netherlands, spoke about hypomyelination and autosomal dominant porencephaly. Some disorders of hypomyelination such as Charcot Marie Tooth involve the connexin genes. Connexins are a family of proteins that are responsible in establishing gap junctions that allow rapid exchange of ions and small molecules (like second messenger molecules). Three connexins, Cn 29, Cn 32 and Cn 46.6, are located on cell bodies and processes of oligodendrocytes and myelin. Cn 32 is most abundant on myelin and Cn 46.6 is mostly present at oligodendrocyte-astrocyte junctions. With a defect in connexin 46.6 myelin deposition becomes insufficient during stress situation. This reveals the importance of interactions between astrocytes and oligodendrocytes. Recently mutations in collagen IV A1 were found responsible for autosomal dominant porencephaly. This disorder is related to a microangiopathy in adulthood and presents with a progressive leukoencephalopathy, calcifications, ischemic and hemorrhagic strokes and cataracts. Collagens IV are ubiquitous proteins in basement membranes including vascular basement membranes. A1 and A2 are most abundant types of collagen IV, and confer strength to basement membranes. Mutations of A1 lead to focal destruction of basement membranes and by way of narrowing of vessels to subsequent ischemic damage. Thus the pathogenesis and molecular biology of leukodystrophies is highly complex and the etiology concerns not only myelin and oligodendrocytes but may include many other white matter components such as gap junctions and connexins.

Dr. Sakkubai Naidu from the Kennedy Krieger Institute, Baltimore, presented her work in the second opinion network. She focused on her observations of Hashimoto's encephalopathy as well as a recently recognized white matter disorder. A patient with Hashimoto's encephalopathy was found to be slightly hypothyroid after a dramatic global decline. Imaging showed changes in the internal capsule and posterior regions of the brain and high levels of thyroid antibodies were

found in serum and led to the subsequent diagnosis. With steroid treatment considerable improvement occurred but behavioral problems persisted. This remains a rare but treatable condition and therefore important to recognize. Dr. Naidu further presented the clinical and imaging findings of a novel white matter disorder presenting with spastic paraparesis and mental deterioration. On MRI these patients show very mild abnormalities in the white matter in addition to thinning of the corpus callosum leading to the descriptive term “disorder of thin anterior corpus callosum with dementia”. Over the past 7 years the genetic basis has now been identified and implicates two separate loci for the disease. This knowledge will help us understand the biological basis of axonal damage and seizures in this disorder.

Dr. Adelene Vanderver from the Children’s National Medical Center in Washington presented her work on defining a clinical biomarker for eukaryotic initiation factor 2B (eIF2B) related disorders. eIF2B is the gene responsible for vanishing white matter disorder (VWMD) and has an important role in protein translation. Due to the clear role of the gene in protein translation Dr. Vanderver has focused on the proteomics of cerebrospinal fluid in VWMD patients. Standard 2D gel electrophoresis of spinal fluid revealed different patterns in transferrin isoforms of VWMD patients compared to controls (this difference was not detected in serum). Further analysis revealed that asialic acid isoforms were decreased in patients with eIF2B mutations. Using this technique all patients with eIF2B mutations could be correctly identified. The technique was shown to be 100% sensitive and 94% specific and furthermore quite cost effective compared to gene sequencing for VWMD (the test costs 12 \$ and can be performed in 2 days). The limitation remains that it is a semiquantitative test.

Dr. Raphael Schiffman from NIH, Bethesda, also introduced a novel leukodystrophy with myelin disorganization and absent or decreased function of the gonads. All patients had a normal early development with onset of ataxia and dementia between 7 and 12 years of age, no spontaneous onset of puberty, tooth abnormalities, decreased tendon reflexes despite normal nerve conduction reflexes and absent or decreased function of the gonads. MRI shows hypomyelination and occasional cerebellar atrophy. Sural nerve biopsies on three patients manifested exactly the same unusual abnormalities: disorganization of the myelin, some axonal loss and inclusions inside the myelin, which resemble cholesterol crystals. Dr. Schiffman emphasized the structural abnormality of peripheral nerves in the presence of normal neurophysiology. Exploring this observation may further help clarify genetic myelin disorders.

Dr. Vladimir Berginer from Beer-Sheva, Israel, presented an overview of cerebrotendinous xanthomatosis (CTX) and described the historic evolution of the disorder from a fatal condition to a condition easily treatable in the early stages. CTX is a lipid storage disease characterized by diarrhea, cataracts, xanthomas, and progressive neurologic dysfunction. The condition was first described in the 1930s when the patients were noted to have xanthomas in the presence of normal serum cholesterol. It was not until 1968 that Menkes discovered that it was not storage of cholesterol but of cholestanol, a compound formed by reduction of cholesterol. Due to the low chenodeoxycholic acid (CDCA) levels Dr. Berginer began to supplement patients with chenodeoxycholic acid and found that long-term treatment normalized bile acid synthesis and plasma cholestanol, and brought marked clinical improvement. He was able to reverse long tract signs, improve gait imbalance and cure seizures without the use of anticonvulsants. Today it is clear that the prevalence of CTX is much higher than previously recognized. There are more than 60 patients in Israel alone and there continue to be many undiagnosed patients. The reported success of treatment emphasizes the importance of early diagnosis, since treatment with CDCA prior to onset of symptoms can prevent disability in CTX.

Dr. Jean de Vellis from UCLA, California, spoke about his research on the mouse model of Canavan's disease (CD). CD is a leukodystrophy characterized a spongy degeneration of the brain, macrocephaly, and hypomyelination. It is caused by a deficiency in aspartoacylase (ASPA), which leads to an elevation in N-acetylaspartic acid (NAA). NAA is one of the most abundant metabolites in the central nervous system. Its chemistry and function is complicated by the fact that it involves three major cell types of the brain: neurons, astrocytes, and oligodendrocytes (OL). Neurons produce NAA that when released is metabolized to N-acetylaspartyl glutamate (NAAG). NAAG itself is cleaved by an enzyme on astrocytes and thereby converted back to NAA and glutamate. Finally, NAA is taken up by oligodendrocytes that metabolize it to aspartic acid and acetate. Large amounts of acetate are required to maintain myelin during development (25% of acetate found in myelin lipids is from this pool). The fact that so many cells are involved has made it difficult to treat CD. Although, NAA is considered a marker for functional neurons in the adult brain its presence has also been identified at much higher levels in OL progenitors and immature OL in culture, while mature OL had undetectable levels of NAA. It appears likely that the lack of ASPA in OL leads to abnormal OL development. Yet the impact of the metabolic derangement upon the different cell types is unclear. Beyond an arrest in development the ASPA knockout mouse model also shows massive cell death in adulthood. Further, active cell renewal has been observed within areas of massive cell death. The triggers for cell death and the function of cell proliferation in CD are areas of active research.

Dr. Shaloni Kumar from UCLA, California spoke about aspects of therapy in Canavan's Disease (CD) and the generation of neural stem cells from the animal model. Aspartoacylase (ASPA) gene delivery attempts in animal models have shown a lowering of NAA and a change in motor function. Yet the sponginess of the white matter, a characteristic of CD remained unchanged even with better viral serotypes and delivery of the gene during early phase of development. The observation of massive cell death in the adult ASPA knockout mouse as well as a concurrent generation of a new wave of cells in white matter led to following therapeutic approach: neural stem cells were isolated from the adult mutants and transplanted into normal newborn rat brains. They showed good migration and differentiated into astrocytes, progenitor cells, and immature OL, but failed to progress to maturation. These observations may help resolve whether the process of hypomyelination or demyelination is a prevalent factor in CD. Furthermore, these preliminary results suggest that the CD mutation may has a broad impact on development and degeneration and disrupts the balance of a complex regulatory system in the brain.

Dr. Wolfgang Koehler from Saxonian hospital, Humbertusburg, presented some late-breaking news on polymorphisms of methionine metabolism in X-linked adrenoleukodystrophy (X-ALD). Within a sample of 86 patients with X-ALD, this genotype was overrepresented in a subgroup of 15 patients with adrenomyeloneuropathy (AMN) that showed cerebral demyelination in comparison to 49 AMN patients without cerebral demyelination, suggesting that methionine metabolism might contribute to the phenotypic variability in X-ALD. This data has yet to be reproduced by other groups but may guide our choice and timing of interventions in the future.