2003 Research Grants

The National MPS Society awarded seven research grants for 2003. Each grant is for $30,000/year for two years. Funding for the grants was provided by the generous donations of our members for syndrome specific research and also by the money raised by the 2002 walk/runs. We are very grateful to the R. A. Bryan Foundation for funding one of the MPS III grants. Twenty-one proposals were received, including 6 for MPS I and 12 for MPS III. We regret that no proposals were received for MPS II, so that grant will not be funded in 2003. Members of our Scientific Advisory Board reviewed the proposals; the final determination of funding was made by the Board of Directors.

"SB (Sleeping Beauty) Transposon-mediated Gene Therapy for MPS I"

Dr. Elena Aronovich, Pediatrics and Institute of Human Genetics, University of Minnesota, Minneapolis, MN

The Sleeping Beauty transposon, a novel, non-viral integrating system for gene transfer, will be tested as a tool for gene therapy of mucopolysaccharidosis type I. Our preliminary data obtained with an MPS VII mouse model indicate the feasibility of this approach. Using the transposon for expression of b-glucuronidase, we achieved enzyme levels that were sufficient to reverse lysosomal pathology in adult mouse livers in a 2-month experiment. Here, we propose to construct therapeutic transposon vectors carrying a human a-L-iduronidase gene that will be injected intravenously into MPS I mice. Correction of a-L-iduronidase enzyme deficiency and lysosomal pathology will be evaluated in mouse livers in a long-term (4 months) study. The biodistribution of the therapeutic transposon will be also determined. The proposed experiments will lay groundwork for future application of SB-mediated gene transfer to treatment of MPS diseases in human patients.

"Intravitreol Gene Therapy in III B Mice"

Dr. Judith Ogilvie, Ophthalmology & Visual Services, Washington University, St. Louis, MO

Mucopolysaccharidoses (MPS) are progressive diseases usually resulting from genetic defects in one of the lysosomal enzymes. Treating the eyes of adult MPS VII mice with virus-mediated gene therapy reduces lysosomal storage in treated eyes and in some parts of the brain. However, the severe systemic disease complicates accurate functional testing. Determining how much function can be recovered after treating established lysosomal storage in the nervous system is important, because most individuals with these diseases are diagnosed only after they begin to

show symptoms. This proposal's goals are to examine whether intravitreal gene therapy improves vision function in MPS IIIB mice, and to lay the groundwork for future studies testing higher brain function. These mice have well-characterized pathology in their eyes and brains. Their longer life-span allows us to follow expression of the therapeutic enzyme longer than in MPS VII mice. Furthermore, this study will show whether transport from the eye into the brain works with a different lysosomal enzyme. Finally, since MPS III diseases predominantly affect the nervous system with mild systemic complications, behavioral tests can be performed at later ages. The proposed studies will provide the necessary preliminary information for designing cognitive function experiments in MPS IIIB mice.

"Gene Therapy in Canine MPS III-B"

Dr. Philippe Moullier and Matthew Ellinwood, Laboratoire de Therapie Genique, Nantes Cedex, France

The focus of this work is the further development of the canine model of MPS IIIB with an emphasis on gene therapy. 1) We propose to treat affected dogs at birth intravenously with gene therapy vectors to evaluate the ability of the normal gene to produce enzyme that is capable of being secreted into the blood and cross correct cells in other parts of the body. While this approach may not directly address the serious issues of brain disease in MPS IIIB, it provides important information about whether the gene is capable of producing normal enzyme that can be taken up by other cells. This is critical information, necessary for effective gene therapy to treat the brain disease seen in MPS IIIB. 2) We will evaluate gene therapy to the brain, via the direct injection of gene therapy vectors or stem cells. 3) Concurrent with these projects will be the continued study of the canine model, with an emphasis on identifying signs of disease that will help evaluation therapy.

"Inhibition of GAG Synthesis as a Therapy for MPS IVA and VI"

Dr. Sharon Byers, Women's and Children's Hospital, North Adelaide, Australia

MPS disorders arise from a deficiency in an enzyme required for the degradation of complex carbohydrate molecules. Children with MPS display symptoms that include but are not limited to reduced height, blindness and brain pathology. Multi-tissue treatment for MPS is the subject of intensive research and centres around increasing the amount of deficient enzyme through enzyme replacement therapy (ERT), bone marrow transplantation or gene therapy. ERT in particular has shown great promise and has reached clinical trial for several MPS. However, some tissues with limited access to the circulation are not amenable to intravenous ERT. These include the brain, cornea and cartilage. Current therapy protocols thus do not address all sites of pathology. In this project therapy will be approached from a different direction. The synthesis of the complex

carbohydrate substrate normally degraded by the deficient enzyme will be reduced to a level that more closely matches the reduced enzyme activity observed in MPS patients, this concept is termed substrate deprivation therapy. Small sugar analogues of glucose and galactose will be synthesized and assessed for their ability to inhibit carbohydrate synthesis. The advantage of this type of therapy is that the inhibitors will equilibrate with cellular pools of carbohydrate throughout the body. All sites of pathology in MPS are thus amenable to substrate deprivation therapy.

"Joint and Bone Disease in MPS VI"

Dr. Calogera Simonaro, Mr. Sinai School of Medicine, New York, NY

This grant proposal will study joint and bone disease in animals with mucopolysaccharidosis (MPS) type VI (Maroteaux-Lamy disease). Our goal is to gain a better understanding of this disorder and to develop new and/or improved therapies. We will specifically study the mechanism(s) causing cell death in cartilage and bone, and the role of inflammation in the disease process. We will also examine enzyme therapy and other therapeutic strategies for these conditions. It is our hope that these studies on animal models of MPS VI will lead to more effective therapy for this and other MPS disorders, particularly in the joints and bones.

"Pathological and Molecular Characterization of Feline Mucolipidosis II - First Model of Human I-Cell"

Dr. Urs Giger, Medical Genetics, University of Pennsylvania, Philadelphia, PA

Mucolipidosis II (ML II), also called I-cell disease, is a unique cellular storage disease leading to severe skeletal malformations, growth and mental retardation, and death within the first decade of life. Although ML II is caused by faulty trafficing of enzymes to reach cellular organelles (lysosomes), it shares many clinical features of the more common forms of mucopolysaccharidoses (MPS). We have established a colony of domestic shorthair cats with naturally-occurring ML II, the first model in which to study this rare storage disease. After we documented the clinical features in cats and mode of inheritance, we propose now to characterize the pathology of ML II in tissues from affected cats and compare the results to the scant information from human patients. Although the deficient enzyme has been identified, the molecular basis remains unknown in affected cats as well as humans. We therefore also propose to characterize feline I-cell disease at the molecular genetic level by sequencing the gene of the normal enzyme and identify the disease-causing mutation in affected cats. Thereby, the knowledge gained in feline ML II will likely further our understanding of this disease in humans and provides the necessary characterization for this animal model to become useful in the development and assessment of the safety and efficacy of novel therapeutic interventions.

Dr. Elsa Shapiro awarded MPS Research Grant

Occasionally the MPS Sociey receives requests for grant funding outside our normal funding cycle. The year we received 2 excellent proposals, and the Board of Directors chose to award a one year grant of $8750 to Dr. Elsa Shapiro at the University of Minnesota for the fellowship of Dr. Kendra Bjoraker. This grant was possible because not all the money allocated for the Partnership Grant was requested.

"Research Training in Psychosocial Development and Quality of Life in MPS Disorders". Dr. Elsa Shapiro, University of Minnesota, Minneapolis, MN Few psychologists have expertise in MPS disorders. As a result, little research has been done on the factors that contribute to quality-of-life and psychosocial outcomes of children with MPS. We need to determine how new treatments (such as enzyme replacement or gene therapies) as well as established therapies such as bone marrow and cord blood transplant might alter quality-of-life and psychosocial status as well as the previously studied cognitive and language development. Also, this will enable us to find ways to improve the quality-of-life and psychosocial outcomes of these children.

We propose to develop a training program at the University of Minnesota to train a neuropsychologist to carry out such research to increase understanding of MPS. We have developed a detailed program curriculum. We are uniquely able to carry out training at Minnesota due to the number of physicians and psychologists here who can provide expert mentorship. In addition to training in the disciplines necessary to carry out this research, the trainee would carry out such a study during the year's work. We hope that training such a person would be a model for other centers to pursue such research and training and would lead to federal funding.

Partnership Grant 2003

The Board of Directors of The National MPS Society approved a 2003 Partnership Grant with Julia's Hope Medical Research Foundation for Sanfilippo Syndrome. A total of 3 grant proposals were received, all of which were for MPS III research. The funding is for one year, with both groups granting $41,000.

"Sleeping Beauty Transgene Vehicle ? Potential New Therapy for MPS III-A"

Dr. John Hopwood, Lysosomal Diseases Research Unit, North Adelaide, Australia.

This grant is a Partnership Grant with Julia's Hope Medical Research Foundation for Sanfilippo Syndrome. The funding is for one year with both groups granting $41,000. The aims of this research project are to utilize a unique and recently described non-viral transposon system (named Sleeping Beauty [SB]) to facilitate gene augmentation therapy in lysosomal storage diseases (LSD) that affect the brain, with the view to preventing or reversing clinical signs and symptoms. We will begin by testing our potential new therapy in the mouse model of MPS III-A. This research will determine the breadth of the effect of the treatment on the brain. As LSD affect widespread areas within the brain, our treatment needs to have access to most of the brain to be effective in reversing the clinical signs and symptoms. The research will also determine whether the clinical manifestations of these disorders can be prevented in newborn animals, delayed/reversed in young animals or reversed in older animals

Reviews of 1st Year Funding awarded in 2003

Elena Aronovich, PhD

Pediatrics and Institute of Human Genetics

University of Minnesota, Minneapolis, MN

"SB (Sleeping Beauty) Transposon-mediated Gene Therapy for MPS I".

The Sleeping Beauty (SB) transposon system created at the University of Minnesota is one of the few non-viral gene therapy systems that are able to integrate genes into human chromosomes. This is especially relevant for treating genetic disorders, such as mucopolysaccharidoses, that require life-long expression of the therapeutic gene. The purpose of this funded project is to see if the SB transposon system can efficiently deliver the ?-L-iduronidase (IDUA) gene to chromosomes in MPS I mice for long-term correction of the disease.

The SB transposon system consists of two parts: the transposon that carries the therapeutic gene and the source of transposase enzyme that cuts the gene out of the plasmid and pastes it into the chromosomal DNA. Without the transposase, the therapeutic gene will still be expressed, but only short-term, since it is unlikely that it will be integrated into the chromosome. We constructed a series of SB transposon vectors that all provided high IDUA activity but differed in the level of the transposase enzyme. The constructs were tested for transposition efficiency in cell culture. The most efficient construct was then injected into MPS I mice that were completely deficient in IDUA activity. The control group of MPS I mice did not get the transposase. The mice were immune-suppressed with the drug cyclophosphamide to allow detection of IDUA, which is recognized as a foreign protein in the MPS I mice. Two other groups of MPS I mice were treated with the therapeutic transposon vectors, but without immune suppression. Blood samples were collected one day after treatment and once every two weeks thereafter. Delivery of the therapeutic transposon was considered technically successful by the observation of plasma IDUA activity >100-fold of wild type levels the day after treatment. In treated MPS I mice that did not receive cyclophosphamide, IDUA activity was undetectable in all mice six weeks after delivery of the therapeutic gene. No IDUA activity was detected in the livers of these mice three months after plasmid administration. In all immune-suppressed mice, the initial plasma activity levels dropped approximately 150-fold over the first two weeks after delivery, but then remained stable in transposase-positive mice (up to five times higher than in the wild-type mice). But in transposase-negative (control) MPS I mice the IDUA levels continued to decline gradually over three months. In the liver, at three months, IDUA activity was detected in both transposase-positive and transposase-negative groups. However, in the former group, these levels were on average four fold higher. The obtained levels of IDUA activity were sufficient to reduce dramatically the number and size of pathologic inclusions in liver, up to their elimination, which was demonstrated by staining with toluidine blue of liver sections.

Thus, in a long-term experiment (three month experiment) a single dose of SB transposon system

resulted in partial to complete correction of IDUA activity in livers of treated adult MPS I mice.

Our future efforts will be to: conduct a longer-term (6-12 month) study of IDUA expression in treated mice, define a pattern of biodistribution of the circulating IDUA enzyme in the treated MPS I mouse organs, and find a way to prevent more efficiently the inhibitory antibody response to the therapeutic IDUA enzyme.

The results of this year's work have been reported at the annual WORLD Lysosomal Disease Research Network Symposium, Minneapolis, Minnesota, May 13-15, 2004:

1. Applications of the Sleeping Beauty Transposon To Lysosomal Storage Diseases Perry B. Hackett, Elena L. Aronovich, Jason B. Bell, Betsy T. Kren, Brenda Koniar, Roland Gunther, Scott McIvor, Chester B. Whitley (platform presentation)
2. Long-term Expression of Sleeping Beauty Transposon in the Murine Models of Mucopolysaccharidosis (MPS) Type VII and Type I Elena L. Aronovich, Jason B. Bell, Lalitha R. Belur, Joel L. Frandsen, Roland Gunther, Brenda Koniar, David C.C. Erickson, Beth Larson-Debruzzi, John R. Ohlfest, R. Scott McIvor, Perry B. Hackett, and Chester B. Whitley

Dr. Judith Mosinger Ogilvie

Ophthalmology & Visual Sciences

Washington University School of Medicine, St. Louis, MO

"Intravitreal gene therapy in MPS IIIB knockout mice"

We have made considerable progress on two goals. First, we have successfully built a breeding colony of MPS IIIB knockout mice. These animals are now available in sufficient numbers for experimental testing.

Secondly, we are making the gene transfer vector. Although Fu et al (Mol Ther 5:42-49, 2002) have constructed rAAV vectors encoding NAGLU cDNAs, we decided to prepare our own vector in order to be able to compare results from this study to those previously reported for AAV-GUSB in MPS VII mice (Hennig et al, J Neurosci, 23:3302, 2003; Hennig et al, Mol Ther, in press). We started with the same backbone previously used for a ?-glucuronidase vector. We then inserted human NAGLU cDNA from pCMV-hNAGLU (a kind gift from Elizabeth Neufeld), excised with EcoRI and ligated into pTR-CAGG?530 in place of GUSB (excised by partial digest). The insert orientation was confirmed by restriction digest as follows:

ENZYME	PREDICTED SIZES	BANDS SEEN
EcoRI	4829, 2481, 1590 bp	5.0, 2.3, 1.6 kB
Sma I	4540, (3221+1390) or (3686+1189)	4.5, 3, ~1.5 kB
Bgl II	4940, (3586+370) or (2286+1515)	5.0, 3.5

Sma I+Not I 4540, (2888+1385) or (3514+760), 172

These results indicate no recombination of inverted terminal repeats and that the NAGLU cDNA is in the correct orientation to produce protein.

In order to determine whether the construct produces active NAGLU enzyme, we transfected four fibroblast cell lines prepared from NAGLU -/- and +/+ littermate tissues according to the Mark Sands' Lab protocol. We tested the following conditions: transfection with 3 dilutions of plasmid containing the AAV genomic construct, control plasmid DNA, medium with no DNA, and not transfected. NAGLU -/- cell cultures showed a slight but clear, dose-dependent increase in positively stained cells in wells that had been transfected with AAV-NAGLU construct. NAGLU enzyme activity levels in medium from transfected cultures were slightly higher than untreated or sham-transfected culture medium for NAGLU-deficient flat cell cultures.

These results indicate that the construct does produce active enzyme and we are now proceeding to make the virus for gene therapy in the MPS IIIB knockout mouse.

Philippe Moullier, M.D., Ph.D., and N. Matthew Ellinwood, D.V.M., Ph.D.

Inserm U 0649-Laboratoire de Th?rapie G?nique, CHU H?tel-Dieu. Nantes, France

"Evaluation of Gene Therapy in the Canine Model of MPS IIIB".

The focus of this grant is the further development of the canine model of MPS IIIB with an emphasis on gene therapy. Broadly speaking there have been two main goals in this grant. The first group of aims involves the construction and analysis of gene therapy vectors to be used in vivo in the canine model that are designed to deliver normal copies of the canine N-acetyl-?-D-glucosaminidase (NaGlu) cDNA. The second group of aims involves further characterization of the canine model so that therapies can be evaluated in a timely manner, obviating the need to assess their impact on clinical signs, which are adult onset in this canine model.

In pursuit of the first set of aims, work is underway on the construction of a gene therapy vector containing the canine NaGlu cDNA in the context of a recombinant adeno-associated viral (rAAV) vector derived from the AAV serotype 2. This vector will be assessed by intracerebral injections in the canine model. In support of the second aim of the grant, work has progressed on a better understanding of the natural history of the disease in the canine model. The canine model of MPS IIIB is unlike other models in two respects. First, combined with the canine models of MPS IIIA, it is among the only large animal models of MPS disorders to show overt neurological clinical signs. Secondly, unlike the clinical signs of MPS seen in other large animal models, these clinical signs are of early adult onset. This latter fact makes it critical that biochemical markers and/or pathological findings which differ between normal and affected dog be identified at a young age. To this end we have pursued analysis of affected dogs looking at histopathological signs of disease, as well as at biochemical changes in the brains of affected dogs. Histological, lesions associated with lysosomal storage can be distinguished in the liver

and kidney of affected dogs as early as 3 months of age. These findings were also seen in affected dogs at six months of age. No findings of lysosomal storage has been seen in the central nervous system of affected dogs at these ages using convention histopathological techniques, however semi-thin section analysis will be pursued. In an effort to find central nervous system biochemical markers associated with disease in the canine model, we have conducted ganglioside analysis of the cerebral gray matter of affected dogs, ages three months to six years of age. This work was done in at the Lyon-Sud Medical School in Lyon, France, in collaboration with Dr Marie T. Vanier and with the assistance of her graduate student Lucie Verot. Gangliosides, some types of which have long been known to accumulate in the brains of patients with some forms of the MPS disorders, were found to be elevated in dogs as early as 3 months of age. The accumulated gangliosides (GM2 and GM3), remain elevated relative to age matched normal controls from 3 months of age onward, and this elevation increases until the end stages of the disease.

In the course of this year of the grant, Dr. N. Matthew Ellinwood, who was funded as a post-doctoral fellow by this grant, was selected to begin an assistant professorship in the Animal Genetics group within the Animal Science Department at Iowa State University, in Ames Iowa. This is primarily a research appointment, and Dr. Ellinwood, whose position begins October 1, 2004, will continue to work on canine MPS IIIB as the major focus of his research. The canine breeding colony, the establishment of which was supported by the National MPS Society, has been housed at the University of Pennsylvania, and is in the process of being transferred to Iowa State University, a process which will be completed this fall. In consideration of Dr. Ellinwood's change of status, it has been proposed to the National MPS Society that the second year of this grant be transferred to Dr. Ellinwood at Iowa State University, where it will serve as the source of a stipend for a post-doctoral fellow in Dr. Ellinwood's research laboratory.

Findings presented above have been presented in abstract form at an NIH symposium on the Glycoproteinoses and Related Disorders (April 2004), and are submitted to the American Society of Human Genetics meeting (October, 2004). All work present has acknowledged the funding support of the National MPS Society.

Dr. S. Byers

Department of Genetic Medicine,

Women's and Children's Hospital, North. Adelaide, South Australia

"Inhibition of glycosaminoglycan synthesis as a therapy for mucopolysaccharidosis type IVA and VI"

The goal of this proposal is to develop and evaluate substrate deprivation therapy for MPS IVA and MPS VI, using small molecular weight inhibitors of glycosaminoglycan synthesis. In the first instance therapy for MPS IVA has been prioritized. One of the obstacles to evaluation of any type of therapy for MPS IVA is the availability of a convenient in vitro system to monitor correction of storage. The most widely used cell type, the skin fibroblast, does not synthesize or

store significant amounts of keratan sulphate and is therefore not suitable for testing therapy regimens. Our first aim was to develop an in vitro assay system. To achieve this, the keratan sulphate containing domain (G1-G2) of the large cartilage proteoglycan, aggrecan, was isolated and cloned into 2 different expression vectors; an HIV-1 based lentivirus (pHIVmpsvG1-G2) and pCDNA3.1v8HisTOPO (pTOPOG1-G2). Normal cells infected with pHIVmpsvG1-G2 expressed low levels of the keratan sulphate domain as determined by Western blot. Work is in progress to optimize expression from both constructs and assess both skin fibroblast and bone osteoblast cells as mediators of expression.

The concept of substrate deprivation therapy and its application to keratan sulphate synthesis has been demonstrated in normal bovine articular cartilage cell cultures. Cartilage chondrocytes produce large amounts of keratan sulphate containing proteoglycans. The addition of either a general inhibitor of glycosaminoglycan synthesis or a small molecular weight inhibitor of keratan sulphate synthesis to cell culture medium resulted in a decrease in the level of keratan sulphate produced. Based on other experiments with different glycosaminoglycan types, we have shown that inhibition of glycosaminoglycan synthesis results in decreased storage of gag degradation products in the appropriate MPS skin fibroblast cells. Similar experiments will be performed with the keratan sulphate inhibitors once we have fully developed our in vitro assay for keratan sulphate storage. Large scale synthesis of the small molecular weight inhibitor of keratan sulphate synthesis has been initiated.

Dr. Calogera M. Simonaro
Department of Human Genetics, Mount Sinai School of Medicine, New York, NY

"Joint & Bone Disease in the Mucopolysaccharidoses: Identification of New Therapeutic Targets & BioMarkers Using Animal Models"

The mucopolysaccharidoses (MPS) are inherited metabolic disorders resulting from the defective catabolism of glycosaminoglycans (GAGs). We previously used MPS animal models to investigate the pathophysiology of the joints and bones, major sites of pathology in these disorders, and found enhanced chondrocyte apoptosis and nitric oxide production associated with TNF-? and Il-1. We now report that the stimulation of MPS connective tissue cells by these inflammatory cytokines causes enhanced secretion of several matrix-degrading metalloproteinases (MMPs). In addition, expression of tissue inhibitor of metalloproteinase (TIMP)-1 was elevated, consistent with the enhanced MMP activity. These findings were not restricted to one particular MPS disorder or species, and are consistent with previous observations in animal models with chemically induced arthritis. BrdU incorporation studies also revealed that MPS chondrocytes proliferated up to five-fold faster than normal chondrocytes, and released elevated levels of TGF to counteract the marked chondrocyte apoptosis and matrix degradation associated with MMP expression. However, despite this compensatory mechanism, studies of endochondral ossification revealed a reduction in chondrodifferentiation in the growth plates. Thus, although MPS chondrocytes grew faster, most of the newly formed cells were immature and could not mineralize into bone. Our studies suggest that altered MMP expression, most likely stimulated by inflammatory cytokines and nitric oxide, is an important feature of the MPS disorders. These data also identify several proinflammatory cytokines, nitric oxide, and MMPs as novel therapeutic targets and/or biomarkers of MPS joint and bone disease. This

information should aid in the evaluation of existing therapies for these disorders, such as enzyme replacement therapy (ERT) and bone marrow transplantation (BMT), and may lead to the development of new therapeutic approaches.

Pathological and Molecular Characterization of

Feline Mucolipidosis II:

Urs Giger, PD Dr. med. vet. FVH

Section of Medical Genetics, University of Pennsylvania

Philadelphia, PA

"Pathological and Molecular Characterization of Feline Mucolipidosis II:

The First Model of Human I-Cell Disease"

Mucolipidosis II (ML II), also called I-cell disease, is a unique cellular storage disease leading to severe skeletal malformations, growth and mental retardation, and death within the first decade of life. Although ML II is caused by faulty trafficking of enzymes to reach cellular organelles (lysosomes), it shares many clinical features of the more common forms of mucopolysaccharidoses (MPS). We have established a colony of domestic shorthair cats with naturally-occurring ML II, the first model in which to study this rare storage disease.

Recently we have documented the clinical features in cats and the autosomal recessive mode of inheritance (Mazrier et al J Heredity 2003) and documented the close homology and minor differences between the disorder in humans and cats. As the pathology is hardly described in humans with ML-II, we were keen to characterize the pathology of feline ML II in tissues from affected cats and compare the results to the scant information from human patients. We have prepared histological preparations from autopsied animals and are analyzing each tissue by Jessica Caverly VMD PhD, a veterinary pathologist who received a Reentry Fellowship from NIH for these studies. Furthermore, we are extracting the various tissues to identify the specific storage material including mucopolysaccharides and gangliosides in collaborations with others. Similarly we have cultured fibroblast from affected cats to further characterize the specific inclusions so classic for I-cell disease. Finally, tissues with specific pathology will also be assessed by electron microscopy.

Although the deficient enzyme has recently been identified in humans, the molecular basis remains unknown in affected cats as well as humans. Through William Canfield at Genzyme we were able to gain access to the sequence of the human enzyme N-acetylglucosamine-1-phosphotransferase, compared that sequence to the recently released canine transferase sequence and have developed primers to amplify the exons from normal and affected cats. We have thus

far about 1kb amplified and sequenced and there seems to be close homology between species. We are also using fibroblast cultures for RT-PCR of the feline cDNA and thereby should be able to get the entire sequence shortly, therefore we are in a good position to characterize feline I-cell disease at the molecular genetic level.

Some of our initial findings were presented at the National MPS Society meeting in Mainz and further collaborations for storage pathology and biochemistry were established. Thereby, the knowledge gained in feline ML II will likely further our understanding of this disease in humans and provides the necessary characterization for this animal model to become useful in the development and assessment of the safety and efficacy of novel therapeutic interventions.

Philippe Moullier, M.D., Ph.D., and N. Matthew Ellinwood, D.V.M., Ph.D.

Inserm U 0649-Laboratoire de Th?rapie G?nique, CHU H?tel-Dieu. Nantes, France

"Evaluation of Gene Therapy in the Canine Model of MPS IIIB".

The focus of this grant is the further development of the canine model of MPS IIIB with an emphasis on gene therapy. Broadly speaking there have been two main goals in this grant. The first group of aims involves the construction and analysis of gene therapy vectors to be used in vivo in the canine model that are designed to deliver normal copies of the canine N-acetyl-?-D-glucosaminidase (NaGlu) cDNA. The second group of aims involves further characterization of the canine model so that therapies can be evaluated in a timely manner, obviating the need to assess their impact on clinical signs, which are adult onset in this canine model.

Dr. John Hopwood

Lysosomal Diseases Research Unit, Women's and Children's Hospital

North Adelaide, South Australia, Australia

The Sleeping Beauty transgene vehicle ? a potential new therapy for MPS-IIIA

The present study is investigating a potential new treatment for lysosomal storage disorders (LSD) that affect the brain, the 'Sleeping Beauty transgene vehicle'. Sleeping Beauty (SB) is able to transport genetic material into cells. We believe that we will be able to use Sleeping Beauty to transport the genetic material into cells that is required to make lysosomal enzymes. In LSD patients, this would mean that cells treated with SB would make lysosomal enzyme 'normally', and these disorders could thereby be treated. In particular, we are excited about the ability of Sleeping Beauty to enter the cells in the brain. The MPS IIIA mouse model is being used in these studies.

Our preliminary studies have focused on the use of SB that has a red fluorescent tag (so that it can be located within the brain) (SB-dsRed), which has been constructed by Professor Clifford Steer and Dr Betsy Kren and colleagues. SB-dsRed has been used in the first instance to obtain some understanding of how SB moves around the brain from the injection site and how many cells it treats ? we can determine this by looking to see how many red cells there are in a section of mouse brain. Ideally, we would like SB to treat every cell within the brain. In the next phase of the study, we will utilise the SB vector that is capable of transporting the genetic material into cells that is required to make the lysosomal enzyme sulphamidase. SB-sulphamidase is being constructed by Dr Kren in Minnesota at present.

We have used the SB-dsRed vector and have undertaken: (1) preliminary studies investigating the potential of Sleeping Beauty as a future long-term treatment for LSD that affect the brain. The first step in this process has been to determine how long SB-treated cells are capable of producing the red fluorescent tag (or in the future, sulphamidase). We would like SB to enable cells to produce lysosomal enzymes on a long-term (years) basis, so that this treatment does not have to be given too frequently. These experiments have been carried out in human unaffected and MPS-IIIA cells grown in culture in the laboratory; and (2) subsequent studies where we injected SB-dsRed directly into the newborn and adult mouse brain to determine where the fluorescent tag is seen within the brain. The findings from this work are discussed below.

1. Our work with cultured unaffected and MPS-IIIA cells indicates that SB is able to enter these cells and result in long-term production of dsRed (or in the future, lysosomal enzymes). We observed the presence of red fluorescent cells for at least five-months in culture (the longest time we have studied thus far). Approximately 1% of either unaffected or MPS-IIIA cells were treated by SB, a figure consistent with that achieved by other researchers in other cell types. Both unaffected and MPS-IIIA cells were used in this study as it was important to establish that SB treats unaffected and MPS-IIIA cells in the same manner.
2. Adult MPS IIIA mice received direct injections of SB-dsRed into the brain, and the location of cells treated by SB was determined by observing sections of brain under a fluorescence microscope. We detected red fluorescent cells for up to six-weeks post-injection near the injection site, as expected, but also in a nearby region of the brain that contains cells capable of dividing throughout the life of the animal. This is an exciting observation as it suggests that SB is able to enter dividing cells, thus increasing the number of 'treated' cells within the brain. Injection of SB-dsRed into the adult mouse brain did not result in any complications, nor was there observable tissue damage and all mice recovered uneventfully from the injection procedure, as indicated by steady weight gain and behavioural observations.

In more recent studies, newborn MPS IIIA mice received injections of SB-dsRed into the ventricles (fluid filled spaces) in the brain and the location of SB-treated cells (as determined by the presence of the red fluorescent protein) was determined two-weeks later. Treated cells were observed in even more widespread areas of brain. These experiments are continuing.

In summary, these preliminary studies pave the way for planned experiments using the SB-sulphamidase vector in MPS-IIIA mice (as a precursor to MPS-IIIA dog and then MPS-IIIA

human studies). We are hopeful that cells in widespread areas of the brain will be treated with SB, thus alleviating the deleterious effects of reduced lysosomal enzyme activity in this disorder.

Reviews of the second year of funding for grants awarded in 2003

Intravitreal Gene Therapy in MPS IIIB Knockout Mice
Dr. Judith Mosinger Ogilvie, Department of Biology, St. Louis University

The objectives of this project were to fully characterize the functional and pathological degeneration of the MPS IIIB mouse model of Sanfilippo Syndrome type B, to determine whether visual function could be restored through intravitreal gene therapy, and to lay the groundwork for rational design of studies to assess function in the central nervous system. Preliminary studies in MPS VII mice with virus-mediated gene therapy resulted in correction of lysosomal storage in treated eyes and in several areas of the brain. However, because of the severity of the disease, we could not test the long-term functional consequences of treatment in these mice.

We have succeeded in achieving the first objective with full characterization of the functional and pathological degeneration of the MPS IIIB mouse. Functional evaluation was performed with electroretinograms (ERGs) of retinas from MPS IIIB mice at 4, 8, 12, 16, 20, 30, 34, and 45 weeks of age. ERG testing of light- and dark-adapted mice produces a characteristic wave-form that allows for identification of deficits in different cell types within the retina. The dark-adapted retinal ERG response is depressed by 5 weeks and becomes progressively less sensitive with increasing age. The diminished sensitivity reflects a loss of rod photoreceptor function that achieves persistent significance after 15 weeks. No significant differences were observed at any age in retinal function after light adaptation indicating a normal level of cone photoreceptor function.

We have performed histological analysis on retinas from MPS IIIB mice at the same time points. At 4 weeks of age, the mutant retina appeared grossly normal, although localized abnormalities were seen in retinal pigment epithelium (RPE) cell shape consistent with loss of cell polarization and/or delamination. By 8 weeks of age, lysosomal storage could be seen in vascular cells and microglia in the inner retina. Occasional pyknotic nuclei could be seen in the outer nuclear layer, which is comprised of photoreceptor cells, and further disruption in the RPE was seen. Photoreceptor degeneration, with shortening of the outer segments and cell loss in the outer nuclear layer, became apparent around 16 weeks with a decrease of 2-4 rows of nuclei by 20 weeks of age. Macrophage-like cells were apparent in the subretinal space between the RPE and photoreceptor outer segments by 16 weeks. Nearly half of the photoreceptor cells were gone by

30 – 34 weeks with only 3-4 rows of nuclei remaining at 45 weeks. Outer segments were further shortened and appeared swollen. Large, round, dense melanosome-like structures were seen in the RPE of mutant retinas, first becoming noticeable in the periphery of the mutant eye as early as 4 weeks, distributed throughout the RPE by 16 weeks, and becoming larger and more prominent by 30 weeks. In mutant retinas, unlike wild type controls, these structures were prominent in the mid to basal cytoplasm and were distinguished by their round shape.

This thorough characterization of the retina of the MPS IIIB mouse model of Sanfilippo Syndrome type B is an important step forward in investigating potential therapeutic interventions for this disease. These results have been presented in abstract form at the Association for Research in Vision and Ophthalmology meeting (Hennig, et al., 2006) and are currently in preparation for publication with additional characterization performed by Dr. Mark Sands and collaborators (Heldermon, et al).

Considerable effort was placed in producing a gene transfer vector. Initial results indicated that our construct produced active enzyme. MPS IIIB knockout mice and wild type controls were injected intravitreally with the vector and functional tests were performed at three time points. Tissue was harvested and processed. Unfortunately, the viral construct did not produce sufficient expression of the NAGLU enzyme to determine whether visual function could be restored through intravitreal gene therapy. Although we were disappointed with this result, other experiments performed concurrently with this work have enabled progress on the third objective. We collaborated with Drs. Mark Sands and Megan Griffey on the ppt1 mouse model of Batten's Disease. Those studies (Griffey, et al., 2005) were successful in establishing the groundwork for the future design of studies to assess the ability of intravitreal gene therapy to improve CNS function in lysosomal storage diseases.

Publications:

Griffey, M., S.L. Macauley, J.M. Ogilvie, M.S. Sands. (2005) AAV2-mediated ocular gene therapy for infantile neuronal ceroid lipofuscinosis. Mol. Ther. 12:413-21.

Heldermon, C.D., Hennig, A., Vogler, C., Ohlemiller, K., Ogilvie, J.M., Breidenbach, A., Herzog, E.D., Sands, M.S. "Characterization of the murine model of Sanfilippo syndrome type B." In preparation.

Hennig, A.K., M. Griffey, M.S. Sands, R.L. Gunkel, M.K. Murphy, J.M. Ogilvie. (2006) Electroretinogram Changes and Retinal Degeneration in Knockout Mouse Models of Four Lysosomal Storage Diseases. ARVO Abstract #5780 accessed at www.arvo.org. presented May 4, 2006 at the annual conference of the Annual Conference of the Association for Research in Vision and Ophthalmology.

SB (Sleeping Beauty) Transposon-mediated Gene Therapy for MPS I

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The Sleeping Beauty (SB) transposon system created at the University of Minnesota is one of the few non-viral gene therapy systems that are able to integrate genes into human chromosomes to provide life-long expression of a therapeutic gene. The purpose of this funded project has been to see if the SB transposon system can efficiently deliver the a-L-iduronidase (IDUA) gene to chromosomes of liver in MPS I mice for long-term expression and correction of the disease. The SB transposon system consists of two parts, the transposon that carries the therapeutic gene and the source of transposase enzyme, which cuts the gene out of the plasmid and pastes it into chromosomal DNA. Without the transposase, the therapeutic gene can be expressed, but generally only short-term, presumably because as an unintegrated episome, it is either lost or recognized as foreign DNA and inactivated.

For this study we chose the very efficient, high-pressure "hydrodynamics-based" DNA injection, which targets the liver. We constructed SB transposon plasmid-based vectors and injected them into MPS I mice that were completely deficient of IDUA activity. Because our goal was in part to determine the efficacy of transposition as a way of providing long-term expression of IDUA protein, control groups of MPS I mice did not receive the transposase. Blood samples for plasma isolation were collected 1 day after treatment and once every 2 weeks thereafter. Plasma IDUA activity reached >100-fold of wild type levels on day 1 following treatment, but was essentially gone in all mice by 4-weeks. IDUA activity was not detected in the liver of mice 3 months after plasmid administration. We examined the duration of the transposon-delivered IDUA gene by PCR and IDUA expression in liver of unaffected mice over 6 months. As in the MPS I mice, plasma and liver IDUA activity reached supra-normal levels on day 1 and remained at this level for the first week, but reduced dramatically by the 2-week time point and by 4 weeks were indistinguishable from background. Notably, the presence of the IDUA transgene mirrored the IDUA activity time-line, i.e., the PCR product from the transgene was not detectable after 2 weeks post-injection. However, transposition was confirmed by an "excision assay" (which detects the PCR product of the plasmid that delivered the IDUA gene to the liver). The excision product was detectable for the first two weeks, but was undetectable thereafter. This suggests that cells that express the IDUA gene are cleared from the liver of treated mice by 4 weeks following injection. Our data suggest induction of an immune response either to the therapeutic protein or/and the cells that express the therapeutic gene.

This conclusion was supported by our observations that in all cyclophosphamide immune-suppressed MPS I mice, the initial 1-day plasma activity levels dropped approximately 150-fold by 2 weeks, but then persisted at detectable levels in some mice. IDUA levels were stable in transposase-positive mice (up to 5 times higher than in the wild-type mice) whereas in transposase-negative (control) MPS I mice IDUA levels declined over 3 months. In the liver, at 3 months, IDUA activity was detected in both transposase-positive and transposase-negative groups. However, in the former group, these levels were on average 4-fold higher. The obtained levels of IDUA activity were sufficient to dramatically reduce the number and size of pathologic inclusions in the liver as demonstrated by toluidine blue staining of liver sections. Thus, with immune suppression, a single dose of the SB transposon system resulted in partial to complete biochemical correction (cure) of IDUA activity in the liver of treated adult MPS I mice.

Our future effort will be directed at achieving systemic delivery of the therapeutic gene and finding a way to prevent immune responses to the therapeutic gene in MPS mice.

The results of this work have been reported in three platform presentations and one poster at international meetings:

American Society of Gene Therapy, 2005 Annual Meeting, St. Louis, MO, June 1-5, Duration of Expression of Sleeping Beauty Transposase by Hydrodynamic Injection of C57/BL6 Mice. Jason B. Bell, Elena L. Aronovich, Brenda Koniar Roland Gunther, Beth Larson-Debruzzi, Chester B. Whitley, R. Scott McIvor and Perry B. Hackett Mol. Therapy, 2005, v.11: S423

Third Annual International Conference on Transposition and Animal Biotechnology, Minneapolis, MN, June 23-24, 2005 : Long-term Expression of Sleeping Beauty Transposon in the Murine Models of Mucopolysaccharidosis (MPS) Type VII and Type I.

Elena L. Aronovich, Jason B. Bell, Lalitha R. Belur, Joel L. Frandsen, Roland Gunther, Brenda Koniar, David C.C. Erickson, John R. Ohlfest, R. Scott McIvor, Chester B. Whitley, and Perry B. Hackett

WORLD Lysosomal Disease Clinical Research Network Annual Symposium 2004, May 13-15, Minneapolis, MN

1. Application of the Sleeping Beauty Transposon to Lysosomal Storage Diseases Perry B. Hackett, **Elena L. Aronovich**, Jason B. Bell, Betsy T. Kren, Brenda Koniar, Roland Gunther, R. Scott McIvor and Chester B. Whitley
2. Long-term Expression of Sleeping Beauty Transposon in the Murine Models of Mucopolysaccharidosis (MPS) Type VII and Type I (poster presentation) **Elena L. Aronovich**, Jason B. Bell, Lalitha R. Belur, Joel L. Frandsen, Roland Gunther, Brenda Koniar, David C.C. Erickson, John R. Ohlfest, R. Scott McIvor, Perry B. Hackett, and Chester B. Whitley

Evaluation of Gene Therapy in the Canine Model of MPS IIIB

N. Matthew Ellinwood, D.V.M., Ph.D., Iowa State University, Ames, Iowa.

The focus of this grant is the further development of the canine model of MPS IIIB with an emphasis on gene therapy. Broadly speaking there have been two main goals in this grant. The first group of aims involves the construction and analysis of gene therapy vectors to be used in vivo in the canine model that are designed to deliver normal copies of the canine N-acetyl-a-D-glucosaminidase (NaGlu) cDNA. The second group of aims involves further characterization of the canine model so that therapies can be evaluated in a timely manner, obviating the need to assess their impact on clinical signs, which are adult onset in this canine model.

In the course of this grant, originally awarded to the Laboratoire de Therapie Genique (Dr. Philippe Moullier), Dr. N. Matthew Ellinwood, who was funded from this grant as a post-doctoral fellow in Nantes, France, was selected to begin an assistant professorship in the Animal Genetics group within the Animal Science Department at Iowa State University, in Ames Iowa. This primary research appointment began October 1, 2004, and Dr. Ellinwood's work will continue to focus on canine MPS IIIB. In consideration of Dr. Ellinwood's change of status, it has been agreed that the second year of this grant be transferred to Dr. Ellinwood at Iowa State University. Furthermore, to ensure that the fellowship be used optimally, Dr. Ellinwood has requested an extension of the second year of the award, so that the fellowship is used for primary research after a suitable candidate can be identified and after Dr. Ellinwood's laboratory and canine colony are settled at Iowa State University.

The canine MPS IIIB breeding and research colony was fully transferred from the University of Pennsylvania in December of 2004, and our first ISU litter was whelped in May 2005. Research collaborations are underway with both a veterinary clinical neurologist and radiologist at the School of Veterinary Medicine at ISU. These efforts will allow for gene therapy experiments and further characterization of the model. In addition the laboratory is now poised to begin gene therapy evaluations, and a post-doctoral fellow, Dr. Chun Coa, has been identified, and will begin work in August 1, 2005.

Work supported by this grant was presented at the American Society of Human Genetics meeting (October, 2004). All work present has acknowledged the funding support of the National MPS Society.

Inhibition of Glycosaminoglycan Synthesis as a Therapy for Mucopolysaccharidosis Type IVA and VI

Dr. S. Byers, Women's and Children's Hospital, North Adelaide, South Australia

The goal of this proposal is to develop and evaluate substrate deprivation therapy (SDT) for MPS IVA and MPS VI. These MPS disorders arise from the deficiency of a lysosomal enzyme required for the degradation of keratan sulphate (KS) or dermatan sulphate (DS) glycosaminoglycan (gag) chains respectively. To be effective, SDT must therefore target the synthesis of these gag chains. By decreasing KS or DS synthesis, the balance between gag production and removal can be redressed in MPS IVA and MPS VI. Thus any remaining patient enzyme activity can more effectively degrade the reduced amount of gag arriving in the lysosome in the presence of inhibitor. We have synthesised a small molecular weight analogue of glucose and assessed its ability to inhibit the synthesis of KS and DS gags in cell culture and compared its effect with a non-specific gag synthesis inhibitor. Both inhibitors decrease gag synthesis in a dose dependent manner when added to the culture medium of normal bovine cartilage cells. Using a combination of size exclusion chromatography and enzyme digestion to identify individual gags, a >50% decrease in the synthesis of the KS-gag containing fraction but only a 15% decrease in the DS-gag containing fraction is observed, implying a differential effect on the two gag populations. Work is currently underway to characterize the size and structure of

KS gags synthesised in the presence of inhibitor. These results offer "proof of concept" that SDT targeting gag synthesis as a treatment for MPS disorders has the potential to be a feasible therapy option.

Joint and Bone Disease in Mucopolysaccharidosis Type VI

Dr. Calogera Simonaro, Human Genetics, Mount Sinai School of Medicine

The overall goal of our research is to fill the void in our understanding of MPS bone and joint disease and to develop new and improved therapies that might benefit MPS patients. We specifically study two animal models with MPS VI, but anticipate that the results obtained can be applicable to the general class of MPS disorders and benefit a wide range of patients.

Our studies carried out over the past five years (funded in part by the MPS Society and published in two peer-reviewed papers) have revealed that glycosaminoglycan GAG accumulation is a direct cause of chondrocyte death in the articular cartilage and growth plates of MPS animals, leading to abnormal matrix homeostasis. This enhanced cell death also triggers a series of signaling events that lead to marked inflammatory disease. Together, these two factors (enhanced cell death and inflammation), lead to the characteristic bone and joint disease in the MPS disorders. In addition, cellular defects associated with the maturation of MPS growth plates are likely contributing to abnormal bone growth.

Enzyme replacement studies in MPS animals and human patients have revealed that chondrocytes in joints and bones are difficult to reach following injection due to the poor vascular supply to these tissues and the fact that the target cells are embedded in a dense, negatively charged matrix. With the availability of enzyme replacement it is important to continue to investigate new approaches for improving enzyme delivery to these critical target tissues. We have modified the charge on these enzymes to make them less negatively charged, so that they might penetrate the cartilage matrix more efficiently and enter chondrocytes. Our data supports the notion that altering the charge might enhance its therapeutic usefulness for cartilage and bone.
Our findings have important implications for the treatment of MPS individuals, as well as for the identification of novel biomarkers to monitor disease progression and therapeutic efficacy.

Pathological and Molecular Characterization of Feline Mucolipidosis II:

The First Model of Human I-Cell Disease

Urs Giger, University of Pennsylvania Research Services

Mucolipidosis II (ML II), also called I-cell disease, is a unique cellular storage disease leading to severe skeletal malformations, growth and mental retardation, and death within the first decade of life. Although ML II is caused by faulty trafficking of enzymes to reach cellular organelles

(lysosomes), it shares many clinical features of the more common forms of mucopolysaccharidoses (MPS). We have established a colony of domestic shorthair cats with naturally-occurring ML II, the first model in which to study this rare storage disease.

Recently we have documented the clinical features in cats and the autosomal recessive mode of inheritance (Mazrier et al J Heredity 2003) and documented the close homology and minor differences between the disorder in humans and cats. Clinical signs seem to be rapidly progressive with leg deformities evident from birth; furthermore these kittens also develop retinal and corneal changes that are being further defined. As the pathology is hardly described in humans with ML-II, we were keen to characterize the pathology of feline ML II in tissues from affected cats and compare the results to the scant information from human patients. We have analyzed histological preparations of various tissues from autopsied animals. Interestingly the storage lesions seem to develop slower and be restricted to specific tissues (Caverly et al in preparation). These tissues will be further assessed by electron microscopy. Similarly, specific storage material including mucopolysaccharides and gangliosides, extracted in collaborations with others, could only be found in a limited number of tissues. Finally, we have cultured fibroblast from affected cats to further characterize the specific inclusions so classic for I-cell disease.

Although the deficient enzyme has recently been identified in humans, the molecular basis remains unknown in affected cats as well as humans. Through William Canfield at Genzyme we were able to gain access to the sequence of the human enzyme N-acetylglucosamine-1-phosphotransferase (GNTPA), compared that sequence to the partial shotgun feline sequence of the transferase. We have completed the sequence of the entire feline GNTPA and characterized the close homology to the human GNTPA sequence and gene structure. Comparing the sequence of affected and normal healthy kittens we have identified a putative disease-causing missense mutation Tcherneva, Seng, Caverly, unpublished). Further studies are in progress to establish a screening test and to characterize the effect on the protein.

Some of our initial findings were presented at the National MPS Society meeting in Mainz and further collaborations for future collaborations have been established internationally. Thereby, the knowledge gained in feline ML II will likely further our understanding of this disease in humans and provides the necessary characterization for this animal model to become useful in the development and assessment of the safety and efficacy of novel therapeutic interventions.