Grants Awarded in 2006

The National MPS Society has awarded $290,000 in new grants for 2006. One grant for MPS II and two grants for general MPS and Related Disease research were awarded. Each grant is for a total of $80,000, $40,000 for each of the two years of funding. An additional $50,000 was awarded to Dr. Brooks to further facilitate his work in MPS III with chaperone therapy.

Dr. Joseph Muenzer was awarded $120,000 in February, 2006 for his work "AAV Gene Therapy for Mucopolysaccharidosis II". Dr. Muenzer will receive $60,000 for each of the two years of funding. Funding for this grant is from an MPS II grant not awarded in 2005 plus additional MPS II research dollars received in 2005.

"AAV Gene Therapy for Mucopolysaccharidosis II"
Dr. Joseph Muenzer, University of North Carolina, Chapel Hill, NC

The focus of our laboratory is to develop new treatments for patients with MPS disorders. A mouse model for MPS II (Hunter syndrome) was created in the laboratory to develop gene therapy procedures to treat both the physical and the central nervous system involvement. The goal of gene therapy in MPS II is to replace the abnormal gene (iduronate sulfatase) by introducing a normal copy into cells. Modified viruses can be used to deliver genetic material into cells. We have created a delivery system using an adeno-associated virus (AAV) which contains a normal copy of the gene altered in MPS II. Our research has shown correction of the liver storage of glycosaminoglycans in MPS II mice after IV administration of these viruses. This research proposal is to expand our studies in MPS II mice to characterize the extent of correction in other tissues and to use newer viruses that may allow better expression of iduronate sulfatase thereby improving the effectiveness of MPS II gene therapy. The successful completion of these studies will generate the animal data needed to submit an application for a human gene therapy clinical trial in MPS II patients.

"AAV-Mediated Gene Therapy of the Hunter Syndrome in the MPS II Mouse Model"
Dr. Maria Pia Cosma, TIGEM, Naples, Italy

Children affected by mucopolysaccharidosis type II (MPS II: Hunter syndrome) lack the activity of the iduronate 2-sulfatase enzyme (IDS). They accumulate compounds in their body that gradually kill their cells and damage all of their visceral organs. At present, efficient cures are not available. A gene therapy approach was initiated to treat this disease in a mouse model of MPSII. The affected mice were injected with viral particles that targeted the liver. High levels of active IDS was produced and secreted into their plasma and taken up by all of the organs. This approach gave important results, as the mice were cured of their visceral defects. Now we plan to dissect carefully the brain defects in the MPSII mouse model. We have already generated data showing that we can deliver the IDS enzyme in the brain after systemic treatment of the MPSII mice. We now aim to design an efficient gene therapy approach to cure the brain defects in the mice thought direct deliver of the viral particles in the cerebrospinal fluid. Finally, our aim is to treat baby MPSII mice. Their liver and muscle will be modified to produce and secrete high

amounts of active IDS into the blood, which can again cross-correct the other damaged organs. If successful, this approach should allow the treatment of the disease at a very early stage, and even before the symptoms are manifested.

"Development of a Therapeutic Bone-Targeting System for MPS"
Dr. Shunji Tomatsu, St. Louis University, St. Louis, MO

The long term goal of this research is to establish a bone-targeting system for enzyme replacement therapy to improve the bone lesions present in the mucopolysaccharidoses (MPS). Current approaches for the treatment of MPS have little or no impact correcting the bone pathology. In this proposal, we will test a newly designed drug delivery system to target bone using an acidic amino acid oligopeptide as a carrier. Modifying the enzyme will allow it to be delivered to the bone more specifically and will enhance the drug effectiveness and reduce the side effects. The enzymes that are deficient in MPS will be tagged with the small acid peptide. The tagged enzymes will be tested on MPS mice models, initially MPS IVA, to evaluate whether it is more effectively delivered to the bone lesions compared to the untagged enzymes. By infusing the deficient enzymes into MPS IVA mouse models weekly for a long term, we can see how effectively they treat the disease and obtain helpful information required to design future human trials possibly applicable to all MPS diseases in which patients suffer from bone lesions. We are currently aiming at the human clinical trial on MPS IVA based upon the result.

"Evaluation of Enzyme Enhancement Therapy forMucopolysaccharidosis Type IIIA"
Dr. Doug Brooks, Children, Youth and Women's Health Services, Adelaide, Australia

Genetic diseases are a fundamental cause of human suffering, depriving many children (up to 2.5% of births) and some adults of a normal existence. Lysosomal storage disorders (LSD) are a group of over 45 different genetic disorders, each involving a defect in lysosomal function. Mucopolysaccharidosis III A (MPS III A) is an LSD that affects mainly children, but also some adults and can result in very severe brain disease. MPS II A is caused by a defect in an enzyme called sulphamidase. We plan to develop a new therapy, which will involve stabilising the patient's mutant sulphamidase protein, to increase the level of enzyme activity and treat the disease. We will test various chemical compounds for their ability to increase the level of sulphamidase enzyme activity in patient cells. The project will potentially lead to the development of a safe, non-invasive therapy to treat this severe disorder.

2006 Grants: First year reviews

Dr. Maria Pia Cosma
"AAV-mediated gene therapy of Hunter syndrome in the MPS II mouse model"
TIGEM, Naples, Italy

Mucopolysaccharidosis type II (MPSII; Hunter syndrome) is a lysosomal storage disorder that arises due to the deficiency of iduronate 2-sulfatase (IDS) enzyme activity. We recently characterized the ids knockout, which shows skeleton deformations and an elevated accumulation of glycosaminoglycans in the urine and in many of organs. In addition, the performance of the knockout mice in the open field and walking pattern tests were severely compromised. We designed an efficient gene therapy approach to treat these MPSII mice using adeno-associated viral (AAV) vectors. AAV2/8TBG-IDS viral particles were administrated intravenously in adult animals. The plasma and tissue IDS activities were completely restored in all of the treated mice, up to nine months after treatment. This rescue of IDS activity resulted in the full clearance of glycosaminoglycan accumulation in the urine and in all of the tissues analyzed, with correction of the skeleton malformations and normalization of the performance in the locomotor tests (Cardone et al. Hum Mol Genet 2006). It is gratefully acknowledged that these studies were made possible through funding provided by the National MPS Society Award 2004. With regard to the current MPS Award 2006, to date, our progress towards the aims proposed is as follows:

Aim 1: Characterization of the brain defects in MPSII mice.
We have performed an initial characterization of the brain defects in the MPSII mouse model and seen a loss of Purkinje cells and cellular vacuolization in different regions of the brain: the hippocampus, thalamus, cerebellum and brainstem. We also noted GAG accumulation within the choroids plexus of the ventricular region. To further characterize the brain abnormalities in MPSII mice, we planned to analyze the morphology and numbers of neurons, astrocytes and oligodendrocyte, using different neural markers. To begin this analysis, control wild-type adult mice and MPSII adult mice, all at 12 months of age, were perfused with PFA and the brains postfixed and embedded in paraffin. Sections were analyzed by immunohistochemical analysis using NeuN as the neuronal cell marker, calbindin as the Purkinje cell marker and GFAP as the astrocyte marker. We detected a decreased number of NeuN-positive neurons in the cortex and almost no
positive calbindin signal in the cerebellum of the MPSII mice, as compared to the wild-type animals. In contrast, we detected an increased positive GFAP signal in the astrocytes of the MPSII brain sections. These preliminary data suggest that neurodegeneration and gliosis occur in the brains of adult MPSII mice.

Aim 2: Development of a gene therapy approach for the treatment of the MPSII pups to anticipate disease manifestation.
Preliminary short-term experiments have indicated that when the idsy/- pups are injected with the AAV2/5CMV-IDS and AAV2/8TBG-IDS vectors into the temporal vein, they show a rescue of IDS activity and a clearance of GAG accumulation in all of the organs analyzed, including the brain. To see whether it is possible to prevent the MPSII phenotype, newborn (2 days after

birth) idsy/- pups received 1x1011 total particles of AAV2/5CMV-IDS, of AAV2/8TBG-IDS or of AAV2/5CMV-EGFP in the temporal vein. We plan to follow the efficacy of the therapy throughout their lifetime. We are measuring IDS activity in the plasma and GAG accumulation in the urine each month. The IDS-injected mice are now 13 months old and show very high levels of circulating enzyme in the plasma, and GAG clearance in the urine, with respect to the control group.

In addition, we planned to test the efficacy of the intra-muscular therapy. For this, we have constructed the AAV2/1MCK-IDS vector. The AAV2/1 serotype in combination with the muscle creatinine kinase promoter (MCK) allows efficient muscle transduction and robust expression of the transgene. We tested the prepared vector in wild type mice. Thus, a group of wild-type animals have been injected in the anterior tibialis with 2x1010 total particles of AAV2/1MCK-IDS. The injected animals were sacrificed one month after the injection and the transduced muscles were harvested. The measured IDS activity was extremely high in the injected muscle, with respect to the activities measured in the control (non-injected) group.

Aim 3: Development of a gene-therapy approach to correct the CNS defects of the MPSII mice. To treat the neuropathological features via systemic delivery of the viral particles, we constructed the AAV2/4CMV-IDS vector and disseminated it into the cerebro-spinal fluid through injection into ventricular region IV. The goal of this approach is to transduce CNS ependymal cells and test if these cells, once transduced, can serve as a source for enzyme secretion into the surrounding brain parenchyma and CSF. A group of with 1x1010 particles of AAV2/4CMV-IDS or with 1x1010 of AAV2/5CMV-GFP viral particles as control. The mice were sacrificed one month after treatment and the brains analyzed. An increased IDS activity was seen in the brain homogenates of IDS-injected mice, with respect to the controls. In addition clearance of GAG accumulation was the brain sections of the treated mice.

Dr. Shunji Tomatsu
"Development of a therapeutic bone-targeting system for MPS"
Department of Pediatrics, Saint Louis University, Pediatric Research Institute

We have finished the untagged enzyme experiment on adult and newborn MPS IVA mice. The tagged enzyme is now under investigation. We summarized the results as follows. In the second year, we will finish the bone-tagged enzymes as well.
Summary of the results:
1) Adult MPS IVA tolerant mouse: The pharmacokinetics and biodistributions were determined for two recombinant human GALNSs produced in CHO cell lines: native-GALNS and sulfatase-modifier-factor 1 (SUMF1) modified GALNS. Preclinical studies of enzyme replacement therapy (ERT) by using two GALNS enzymes were performed on MPS IVA mice.
The half-lives in blood circulation of two phosphorylated GALNS enzymes were similar (native, 2.4 min; SUMF1, 3.3 min). After intravenous doses of 250 units/g body weight were administered, each enzyme was primarily recovered in liver and spleen, with detectable activity in other tissues including bone and bone marrow but not in the brain. At 4 h postinjection, enzyme activity was retained in the liver, spleen, bone, and bone marrow at levels that were 20% - 850% of enzyme activity in the wild-type mice. After intravenous doses of 250 units/g of native

GALNS, 250, 600, or 1,000 units/g of SUMF1-GALNS were administered weekly for 12 weeks, MPS IVA mice showed marked reduction of storage in visceral organs, bone marrow cells, osteoblasts, osteocytes, ligaments, and periosteum. A dose-dependent clearance of storage material was observed including brain and cartilage cells although the heart valves were refractory and variable. The blood KS level assayed by tandem mass spectrometry was reduced nearly to normal level. These preclinical studies demonstrate the storage clearance of tissue and blood KS by administered GALNS, thereby providing the in vivo rationale for the design of ERT trials in MPS IVA. Preliminary results by using the bone-targeting enzymes showed that intravenous doses of 250 units/g of the bone targeting GALNS was the same effective as 1,000 units/g of SUMF1-GALNS was used.

2) Newborn MPS IVA knockout mice:
We have used knock-out MPS IVA mice to assess the effects of long-term enzyme-replacement therapy initiated at birth. MPS IVA mice received weekly i.v. injections of 250 units/g body weight recombinant human native or SUMF1-GALNS until 14 wk of age. Either GALNS is able to reach brain and bone until the blood-brain barrier completely closes at 10–14 d of age and avascular region in cartilage cell layer appears. MPS IVA mice that were treated from birth demonstrated near normalization or complete reversal of lysosomal storage in most tissues including bone marrow, bone (osteocytes, osteoblasts, periosteum, and cartilage), ligaments, and heart valves. Nearly absence in storage vacuoles in cells of the CNS in MPS IVA mice treated from birth was also observed. MPS IVA mice treated from birth kept normal level of serum KS significantly lower than untreated MPS IVA mice cells. Preliminary results by using the bone-targeting enzymes showed that intravenous doses of 250 units/g of the bone targeting GALNS was the same effective as native or SUMF1-GALNS was used.
These data suggest that enzyme that enters the brain and the cartilage in the first few weeks of life, before the blood-brain barrier and cartilage cell layers mature, is able to protect against accumulation of storage material in MPS IVA mice.

Dr. Doug Brooks
"Evaluation of Enzyme Enhancement Therapy for Mucopolysaccharidosis Type IIIA"
Women’s and Children’s Hospital
North Adelaide, South Australia Australia

Genetic diseases are a fundamental cause of human suffering, depriving many children (up to 2.5% of births) and some adults of a normal existence. Lysosomal storage disorders are a group of over 45 different genetic disorders, each involving a defect in lysosomal function. Mucopolysaccharidosis IIIA is a lysosomal storage disorder that affects mainly children, but also some adults and can result in very severe brain disease. Mucopolysaccharidosis IIIA is caused by a defect in an enzyme called sulphamidase. We plan to develop a new therapy, which will involve stabilising the patient’s mutant sulphamidase protein, to increase the level of enzyme activity and thus treat the disease. To increase the stability of the mutant sulphamidase protein we will evaluate small molecule compounds called chemical chaperones that either interact with the outside of the protein or help stabilise the protein from within, by interaction with either the core of the molecule or the active site of the protein.

In October 2006 we commenced this project by appointing an experienced research officer for this research project. Because of the drive to develop a new therapeutic strategy for mucopolysaccharidosis IIIA, we first set about validating all of the assay systems that would be required to convince a regulatory authority of the soundness of any data generated. We also cultured a number of cell lines expressing point mutations in CHO-K1 cells and selected fibroblast cell lines that would be appropriate for evaluating the action of chemical chaperones. These cell lines will be used to show increased protein stability and amount of protein/activity in response to chemical chaperone treatment. These initial objectives were completed early in 2007 and showed that the sulphamidase assays were both accurate and not subject to significant variation.

We have now commenced a preliminary screen of the compounds proposed as chemical chaperones. In previous work, we showed that protein stabilisers like glycerol and trehalose (that act on the outside of a protein) can improve the yield of mutant protein and activity, but for sulphamidase we have seen no affect in expression cell lines that had different point mutations. We then evaluated several compounds that would be expected to bind to the inner core of the molecule, to help stabilise the mutant sulphamidase protein. We evaluated glucosamine (a low affinity inhibitor of sulphamidase) and glucosamine N-sulphate and showed some variable increases in the sulphamidase protein. This may indicate a partial effect, or that the conditions that we used were not optimal and we are currently pursuing this line of investigation. Finally, we have just evaluated another different type of inhibitor and have some indications of a very promising result. Thus, we believe that we are on target to achieve the following two scientific aims later in 2007:

Identification of a chemical chaperone that will enhance the level of mutant sulphamidase protein and enzyme activity in cultured cells.
Demonstrate that chemical chaperone treatment can correct the MPS IIIA biochemical storage defect, in vitro.

2006 Grants: Second year reviews

Dr. Maria Pia Cosma
TIGEM, Naples, Italy
"AAV-Mediated Gene Therapy of the Hunter Syndrome in the MPS II Mouse Model?
Received 7-08
Mucopolysaccharidosis type II (MPSII; Hunter syndrome) is a lysosomal storage disorder that is due to a deficiency of the iduronate 2-sulfatase (IDS) enzyme activity. We have characterized the ids knock-out, which shows skeleton deformations and an elevated accumulation of glycosaminoglycans (GAGs) in the urine and in many organs.

More recently, we have started the characterization of the brain phenotype of the knock-out mice. Control wild-type mice and three-month-old adult MPSII mice were analyzed by immunohistochemistry using NeuN as a neuronal marker, calbindin as a Purkinje cell marker, and GFAP as an astrocyte marker. Furthermore, we also tested ubiquitin abundance as a marker for neurodegeneration. In the MPSII mice, we detected a decreased number of NeuN positive neurons in the cortex and a strong reduction in the Purkinje cells in the cerebellum, according to the reduction in the calbindin signal. In contrast, we detected an increase in the GFAP signal and an increase in the ubiquitin-positive signal in the neurons of the MPSII brain sections, with respect to the wild-type mice. These data suggest that neurodegeneration and astrogliosis occurs in the brain of the adult MPSII mice.

We have also performed neurobehavioural tests to analyze the impairments of selected behavioural domains. Groups of control wild-type and MPSII mice of 4.5 and 5.5 months were analyzed. The results have revealed that MPSII mice have an abnormal fear response in the elevated plus maze, spending longer times on the open arms than the control mice, and a memory deficit in the novel-object recognition test, exploring novel and familiar objects equally. These findings show that the emotional and cognitive behavioural domains are impaired in the mutant mice, which are likely to be the behavioral domains that are homologous to those affected inHunter patients.

Regarding therapeutic strategies, we have already designed an efficient gene therapy approach to treat these MPSII mice using adeno-associated viral (AAV) vectors. AAV2/8TBG-IDS viral particles were administered intravenously in the adult MPSII mice. The plasma and tissue IDS activities were completely restored in all of the treated mice up to thirteen months after treatment. This rescue of IDS activity resulted in full clearance of GAGs accumulation in the urine and in all of the tissues analyzed, with correction of the skeleton malformations and normalization of the performance in the locomotor tests (Cardone et al. Hum Mol Genet 2006).

We have now performed systemic delivery of AAV2/5CMV-IDS vectors, with the aim of treating both visceral and CNS defects and to anticipate disease manifestation. We have carried out experiments where we have treated MPSII pups at the newborn stage. We injected groups of MPSII pups in the temporal vein with 1x1011 total particles of AAV2/5CMV-IDS. The mice were sacrificed 1 month and 1.5 years after this injection. The activities of IDS in all of the tissues reached levels that were higher then those in the group of non-treated MPSII mice.

Remarkably, IDS activity was also increased in the brains of the treated mice. The GAG accumulation analyzed in sections was fully cleared in the visceral organs and also in all of the brain areas analyzed (choroid plexus, meninges, cerebellum, cortex) in both groups of treated mice (1 month and 1.5 years after injection). Finally, immunohistochemistry using an anti-GFAP antibody showed a clear amelioration of the astrogliosis phenotype in both groups of treated mice. These data demonstrate that systemic administration of viral vectors carrying the IDS enzyme can ameliorate both the CNS and the visceral symptoms of MPSII mice.

In addition, we are developing a direct CNS-therapy approach. A group of MPSII mice was injected at three months of age with 1x1010 particles of AAV2/4CMV-IDS, or with 1x1010 of AAV2/5CMV-GFP viral particles as the control. The goal of this approach is to transduce CNS ependymal cells and determine if once transduced, these cells can serve as a source for enzyme secretion into the surrounding brain parenchyma and the CSF. The mice were sacrificed 1 month after treatment and the brains were analyzed. Increased IDS activity was seen in the brain homogenates of these IDS-injected mice, with respect to the controls.

Dr. Joseph Muenzer
University of North Carolina School of Medicine, Chapel Hill, NC
"AAV Gene Therapy for Mucopolysaccharidosis II? *review not received*

Dr. Shunji Tomatsu
Department of Pediatrics, Saint Louis University, St. Louis. MO
"Development of a Therapeutic Bone-Targeting System for MPS? *review not received*

Dr. Doug Brooks
Women's and Children's Hospital, North Adelaide, South Australia, Australia
"Evaluation of Enzyme Enhancement Therapy for Mucopolysaccharidosis Type IIIA?
received 11-08
Mucopolysaccharidosis IIIA (MPS IIIA) is a lysosomal storage disorder that primarily affects the central nervous system, leading to progressive loss in brain function. MPS IIIA is caused by a defect in the activity of an enzyme called sulphamidase. Most patients with MPS IIIA have sulphamidase present, but it is either in an amount that is insufficient to enable it to carry out its normal function, or it is in a form that is not active. With the generous support of The National MPS Society this project sought to evaluate a new therapy for MPS IIIA, which centres on the concept of using chemicals to stimulate [or 'enhance'] the sulphamidase that is present in patients and guide [or 'chaperone'] it through a series of cellular processes to enable therapeutic benefit. We have investigated a range of compounds or 'chemical chaperones' that can potentially interact with sulphamidase to increase its stability and function.

The main aim of this two year project was to test a number of possible chemicals for their capacity to enhance the activity of sulphamidase present in cultured cells, enabling us to identify an optimal compound for further testing in an animal model of MPS IIIA.

In the first year we screened a number of potential chemical chaperones in human MPS IIIA cultured hamster ovary cells that expressed different mutations [the inherited mistakes] that cause MPS IIIA. Compounds such as glycerol and trehalose that tend to act as general stabilisers

showed little or no effect on enhancing the activity of the sulphamidase present in these cells. We also tested the compounds ortho- and meta-vanadate, which resemble a specific stage in normal substrate [the material stored in MPS IIIA patients] breakdown but these also showed little or no effect. Next, we evaluated a panel of six compounds that can bind to the active site of sulphamidase. Several of these, including glucosamine, had no effect, but two were found to have an enhancement effect on sulphamidase in cultured cells.

In the second year these two candidate compounds were evaluated in MPS IIIA mouse and human cultured skin cells and showed similar results. Unfortunately, the project was interrupted for six-months at this stage due to the departure of the scientist working on the project. In recent experiments, we observed a dramatic enhancement in sulphamidase activity in cells treated with one of the two candidate compounds, but this effect was not reproducible, suggesting that either the chemical dose or experimental conditions were not optimal. We are continuing to optimise the dosage/conditions for this test compound to enable the evaluation of this or a related compound in animal model experiments.

We thank the Society for its support of this project.