

2007 Research Grants

The National MPS Society has awarded \$508,000 in new grants for 2007. Drs. Biffi, Haskins and Simonaro were awarded the general research grants of \$100,000 total. Dr. Fuller was awarded the \$90,000 MPS II grant, and Dr. Mellon was awarded the MPS III grant for \$80,000. Each grant is for two years, and the researchers will receive half of the total each year.

This year we collaborated with two foundations to offer partnership grants. In May, in partnership with the Ryan Foundation, we awarded \$19,000 to Drs. Katherine Ponder and Mark Haskins for their work in "Retroviral vector-mediated gene therapy for MPS I". The second partnership grant of \$19,000 will be offered in conjunction with ISMRD (International Society for Mucopolysaccharidosis and Related Diseases). We will report the details of that grant in our next newsletter.

Drs. Katherine Ponder and Mark Haskins
Partnership Grant with the Ryan Foundation
"Retroviral vector-mediated gene therapy for MPS I"

Washington University School of Medicine (Dr. Ponder)
St. Louis, MO

University of Pennsylvania, School of Veterinary Medicine (Dr. Haskins)
Philadelphia, PA

Mucopolysaccharidosis I (MPS I) is a lysosomal storage disease caused by deficient α -L-iduronidase (IDUA) activity, which results in the accumulation of the glycosaminoglycans heparan and dermatan sulfate. The severe form, known as Hurler syndrome, causes bone and joint abnormalities, pulmonary and cardiac disease, hearing and visual deficiencies, mental retardation, and death around age 5 if untreated. Hematopoietic stem cell transplantation can reduce some manifestations, but has a 15% mortality rate, costs \$130,000, and requires a compatible donor. Enzyme replacement therapy can also reduce some symptoms, but costs over \$500,000 per year for an adult, requires a weekly infusion, and is not available to all patients. The development of an effective and safe gene therapy for MPS I could have a dramatic positive impact on the lives of patients and the families that care for them. We previously demonstrated that neonatal intravenous injection of a gamma retroviral vector (γ -RV) with an intact long-terminal repeat (LTR) expressing canine IDUA had a truly remarkable effect in both mice and dogs with MPS I, with elimination or reduction in all major clinical manifestations. This was due at least in part to efficient transduction of liver cells, which secreted mannose 6-phosphate (M6P)-modified IDUA into blood, which diffused to other organs and was taken up via the M6P receptor. There was also some transduction of blood cells and an undefined cell type in brain, which may have contributed to the therapeutic response. Although no tumors developed in mice or dogs with this approach, the risk of insertional mutagenesis with an LTR-intact vector is a concern. Another problem is that administration of this vector to adult MPS I mice or newborn MPS I cats resulted in a potent cytotoxic T lymphocyte (CTL) response that destroyed transduced cells. The aims of this project are to: 1) reduce the risk of insertional mutagenesis by developing a self-inactivating γ -RV with a deletion in the enhancer of the 3' LTR; 2) attempt to prevent an immune response by avoiding expression in antigen-presenting cells; and 3) analyze

the duration of efficacy and evaluate for toxicity in a long-lived large animal model (dog). If successful, this study may hasten the development of a simple and effective treatment for newborn patients that will reduce or prevent the devastating clinical manifestations of MPS I.

Dr. Alessandra Biffi

"Novel efficacious and safe gene therapy approaches for the treatment of MPS I"

San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET)

Milano, Italy

Type I Mucopolysaccharidoses (MPSI) is a lysosomal storage disorder (LSD) due to the inherited deficiency of α -L-iduronidase (IDUA) and the resulting accumulation of its toxic substrates in many organs. Among MPSI clinical variants, the Hurler syndrome is fatal in childhood, and represents the form with the higher need for the development of new efficacious therapies, capable of alleviating all disease-related symptoms. Indeed, despite several experimental therapies have been tested both in MPSI animal models and in patients, no efficacious treatment is currently available for the cure of Hurler syndrome. This lack of efficacy is likely due to the difficulty of providing sufficient amount of the functional IDUA to all disease sites, including the brain, in the absence of toxicity. Therefore, the main goal of the project is the identification of a novel gene therapy strategy capable of efficiently deliver therapeutic levels of functional IDUA enzyme to all disease sites of MPS I mice, and of correcting disease manifestations, in the absence of toxicity. To this goal, based on our expertise in other LSD models, we will compare two gene therapy protocols based on advanced generation viral vectors, which might over-come the major limitations of currently available therapies. This work will allow us to identify and further develop towards clinical application the most promising and efficacious gene therapy strategy for the treatment of MPSI.

Dr. Mark Haskins

"Lentiviral Vector Therapy for Canine MPS VII"

University of Pennsylvania, School of Veterinary Medicine

Philadelphia, PA

Based upon experiments in mice, a clinical trial has been approved for our collaborator, Mark Sands, PhD, to use a lentiviral vector containing the human gene for the enzyme that is deficient in mucopolysaccharidosis VII to treat bone marrow cells in culture and then return them to the children with MPS VII. Currently, the clinical trial is on hold while Dr. Sands collects more safety data for the FDA. We have a well-characterized dog model of MPS VII and believe it is essential to test the safety and efficacy of this therapy in MPS VII dogs prior to its use in children. We also have successfully treated MPS VII dogs intravenously with a retrovirus vector at three days of age dramatically improving the skeletal, ocular, and cardiac lesions. Five treated dogs are currently more than 6 years post-treatment and are being maintained to evaluate possible long-term side effects of therapy, together with four dogs treated by intravenous, neonatal adeno-associated virus vector gene therapy.

Dr. Calogera Simonaro

"Pathogenesis and Treatment of the Mucopolysaccharidoses"

Mount Sinai School of Medicine
New York, NY

The past decade has witnessed remarkable advances in the understanding and treatment of the MPS. However, despite these advances, major challenges remain. For example, although enzyme replacement therapy (ERT) has recently become available for several of these disorders, it is extremely expensive and requires life-long infusions of recombinant enzyme. ERT also has very limited effects on the bones and joints, major sites of disease in MPS patients. Our laboratory has been using MPS VI animal models to study the mechanism of disease in bones and joints, as well as to evaluate new approaches to treatment. This research has led to a better understanding of the specific changes that occur in these tissues, facilitating the future design of more effective therapies. In the current proposal we will extend these findings and pursue three aims. In the first we will continue to investigate the mechanism by which GAG storage leads to bone and cartilage destruction using cells from MPS VI rats. In the second we will obtain fluid from the joints of MPS VI cats, and measure the levels of several proteins to see if they are abnormally expressed. We will determine the level of these proteins as a function of age, and evaluate whether they can be used to predict the severity of disease and/or the outcome of treatment in the bones and joints (i.e., biomarkers). In the last aim we will use MPS VI rats to evaluate the effects of two clinically available "anti-inflammatory" medications on the progression of disease, as well as one experimental medication that targets a pathway we have found abnormal in MPS VI cells. If we obtain evidence in the rats that such therapies are effective, in the future these approaches could be evaluated in MPS patients, alone or as adjuncts to ERT.

Dr. Maria Fuller

"Membrane microdomains and improved clinical management for the mucopolysaccharidosis"

Children, Youth and Women ™s Health Service
North Adelaide, SA, Australia

The mucopolysaccharidoses (MPS) are chronic progressive genetic diseases that generally affect young children. Symptoms are debilitating and progressive, and include heart and breathing difficulties, skeletal deformity and brain degeneration. The MPS result from the progressive storage of waste in a component of each cell known as the lysosome. In affected children, the accumulation of this waste interferes with each cell ™s normal functioning and leads to the deterioration and death of cells, organs and tissues. There are no cures for MPS and current treatment options are not without their limitations. Although the underlying genetic defects have been determined for many MPS, the disease process remains poorly understood. The diverse array of clinical symptoms in MPS suggests that many cellular processes are altered. A major one is likely to be the fat composition and distribution in cells. Fats have been shown to be altered in the MPS and this project proposes to examine the types of fats that are altered and their location in the cell. Once we understand the changes in fats, we will attempt to correct these changes using conventional drugs and fatty acid manipulation. Successful studies performed in

cells in this project will pave the way for further studies in animal models to see if the pathology in MPS can be treated with diet and drugs.

Dr. Synthia Mellon

"Neurosteroid treatment of MPS IIIA"

University of California, San Francisco

Department of Obstetrics, Gynecology & Reproductive Sciences

San Francisco, CA

We have identified a potential treatment for a lysosomal storage disorder that involves a class of biological compounds called neurosteroids. These compounds are synthesized in the brain in a developmentally programmed fashion. Among their many effects, they have effects on development of new neurons, survival of neurons, protection against toxicity to neurons. We showed that treatment of a mouse model of the lysosomal storage disorder Niemann Pick Type C (NP-C) with the neurosteroid allopregnanolone doubles lifespan, delays loss of motor function, and rescues neurons that die in NP-C. We now have preliminary data in MPS IIIA mice that a similar treatment with allopregnanolone will enhance lifespan, delay loss of motor function, increase muscle strength, and reduce aggressive behavior. We now propose to expand these studies to include more mice to assess the effect of allopregnanolone treatment on MPS IIIA 1. longevity 2. locomotor function 3. neuronal survival 4. neuronal storage and 5. begin to assess peripheral markers of disease progression and effective allopregnanolone treatment. Successful completion of these aims should provide preliminary data for submission of a larger grant to the NIH.

2007 Grants: First year reviews

Dr. Calogera M. Simonaro

Mt. Sinai School of Medicine, New York, NY

“Pathogenesis and Treatment of the Mucopolysaccharidoses

Received 7-08

The underlying premise of our research is that despite some successes, new treatment strategies are needed to replace, or more likely augment, existing approaches for the MPS disorders. This is particularly true for the bones and joints. In order to develop such approaches, as well as to identify new biomarkers for these diseases, a better understanding of the disease mechanism at the sites of pathology is necessary. Due to the limits of human experimentation, this can best be accomplished using animal model systems.

Based on our preliminary findings, we have chosen to evaluate therapeutic strategies that slow or prevent the pro-apoptotic and pro-inflammatory effects of GAG storage (see below). We have shown that inflammation is a major component of the MPS disorders, although to date the effects of anti-inflammatory medications on these diseases have not been systematically evaluated.

During the past year we have further characterized the inflammatory disease that occurs in MPS VI and other MPS animal models. In addition, we have identified many biomarkers that can be used to monitor disease progression and treatment. One new paper describing these findings has been published this past funding period (Simonaro et al. Am. J. Path. 172, 2008), which represents the third in a series describing the mechanism of joint disease in MPS. [complete review with figures](#)

Dr. Alessandra Biffi

San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET)

Milano, Italy

“Novel efficacious and safe gene therapy approaches for the treatment of MPS I”

Received 7-08

The pipeline of the proposed project consisted in the comparison of the long-term efficacy in alleviating disease manifestations in the MPSI mouse model of three different therapeutic approaches:

- o Allogeneic hematopoietic stem cells transplantation (HSCT) from wild type donors (the treatment currently available for Hurler patients at disease onset);
- o Hematopoietic stem cell (HSC) gene therapy using a lentiviral vector (LV) encoding the human IDUA enzyme;
- o Liver-directed gene therapy using LV encoding the functional IDUA enzyme with the addition of miRNA sequences de-targeting expression from antigen presenting cells (APCs).

To this aim, during the 1st year of funding we focused on:

1. MPS I colony characterization

The MPSI colony was fully established in our animal facility. We applied different relevant outcome measures to MPSI affected homozygous, as well as to unaffected litters, including:

- evaluation of residual IDUA activity in relevant tissues, including brain, liver, kidney, bone marrow;

- pQ-CT, to measure mineral density of long bones (a significantly different density was measured in affected vs unaffected MPSI mice);
- whole body CT, to macroscopically evaluate the skeleton (significant abnormalities have been observed in affected MPSI mice, including reduced long bones length, increased bone diameter, facial abnormalities; all these alterations are currently being quantitatively evaluated);
- CT of the chest, which revealed increased size of the heart; eco-cardiography will be performed to assess the heart functional impairment;
- complete pathology for scoring lysosomal distention in relevant organs, including brain, liver, kidney, bone marrow.

This preliminary evaluation was fundamental for defining proper readouts for our long-term efficacy studies.

2. Feasibility and safety of LV-mediated IDUA over-expression in human HSC

We constructed and produced LV encoding the human IDUA under the control of the human PGK promoter, with the addition of the W-PRE element to increase transgene expression (PGK.IDUA.LV). This vector was used to transduce human (CD34+, from normal cord blood) and murine (lineage negative selection from the bone marrow of both wild type and MPSI donors) HSC, according to already established protocols. Transduction allowed sustained IDUA expression at supra-physiological levels (up to 20 fold above basal levels). IDUA over-expressing cells retained a normal proliferation and differentiation capacity in clonogenic assays in vitro. Further, upon transplantation into adequate models (irradiated MPSI mice for murine HSC, and immunodeficient Rag2^{-/-} gamma chain ^{-/-} mice for human HSC), IDUA over-expressing cells demonstrated long-term engraftment and repopulation potential. Overall, these data demonstrate the feasibility and safety of LV-mediated IDUA over-expression in HSC.

3. Generation of the experimental groups for long-term efficacy evaluation:

Group A: Allogeneic HSCT from wild type donors

HSC were isolated by lineage negative selection from 6-8 weeks old wild type littermates and transduced with control LV encoding GFP under the control of the human PGK promoter, and then transplanted into lethally conditioned homozygous defective MPSI mice, at 2 months of age, to determine the possibility to achieve prevention/correction of disease manifestations. As we already demonstrated that the extent of tissue macrophages and microglia replacement is not affected by the conditioning regimen applied prior to the transplantation, we lethally irradiated recipient mice. The engraftment of donor-derived, GFP transduced cells measured by FACS analysis on peripheral blood mononuclear cells was above 70% in all transplanted animals. A cohort of 15 transplanted animals is currently available for efficacy evaluations. Efficacy of the treatment will be evaluated on 10 months old treated mice, looking at prevention and correction of functional, biochemical and histopathological abnormalities.

Group B: HSC gene therapy with IDUA LV

We transplanted HSC from homozygous defective MPSI mice, transduced with PGK.IDUA.LV, into lethally conditioned, 2 months old IDUA^{-/-} mice. IDUA expression levels measured on the in vitro progeny of transduced HSC by 4-methylumbelliferyl assay showed sustained IDUA over-expression (above 20 fold the basal levels) and vector content was quantified by LV-specific quantitative PCR analysis (5-6 LV copies/genome). A cohort of 20 transplanted animals is currently available for efficacy evaluations. IDUA specific activity will be measured on cell

lysates from PBMC, starting from 8 weeks after the transplant. Efficacy of the treatment will be evaluated on 10 months old treated mice showing IDUA activity reconstitution above wild type levels in PBMC, looking at prevention and correction of functional, biochemical and histopathological abnormalities. Comparison with the outcome of allogeneic HSCT (group A) and with MPSI mice transplanted GFP transduced IDUA^{-/-} HSC will be performed, in order to assess the therapeutic role of enzyme over-expression in the HSC progeny.

Group C: Liver-directed gene therapy with miRNA regulated LV

We constructed and produced LV encoding the IDUA cDNA under the ET or the PGK promoters with and without the addition of target sequences for the mir142-3p, which is robustly expressed in hematopoietic cells, such as. During the next few months, two months old homozygous defective MPSI mice will be injected intravenously with 5×10^8 IU of the tagged LV.ET.IDUA.142-3pT or control LV.ET.GFP.142-3p. ELISA will monitor appearance of neutralizing anti-IDUA or anti-GFP antibodies starting from 7 days after vector administration. Treated animals lacking evidence of antibody production and controls will be evaluated at 10 months of age for prevention and correction of functional, biochemical and histopathological abnormalities and for vector content in affected organs by LV-specific quantitative PCR.

Dr. Mark Haskins

**School of Veterinary Medicine, University of Pennsylvania
Philadelphia, PA**

“Lentiviral Vector Therapy for MPS VII”?

Received 7-08

Our initial experimental plan was based upon experiments in mice, from which a clinical trial had been approved for our collaborator, Dr. Mark Sands, to use a lentiviral vector containing the human gene to treat MPS VII bone marrow cells in culture and then return them to the children with MPS VII. Over the past year, we transplanted seven dogs with gene modified autologous bone marrow. Three control dogs received cells expressing the marker GFP and four MPS VII dogs received cells expressing beta-glucuronidase (GUSB). Briefly, stem cells were isolated from bone marrow aspirates and exposed to recombinant lentivirus supplied by Dr. Sands either overnight or in two cycles over 48 hours. The transductions took place in serum free conditions and the cells were supplemented with cytokines. For the control dogs, $9.7 \pm 2.7\%$ of the cells were determined to be GFP positive by flow cytometry and $0.18 \pm 0.08 \times 10^6$ cells/kg were returned to the donor dogs. The MPS VII dogs received $1.51 \pm 1.27 \times 10^6$ cells/kg, and $30.1 \pm 3.3\%$ of those cells expressed GUSB histochemically. No significant number of GFP positive cells was detected in peripheral blood from the control dogs. For the MPS VII dogs, where flow was unavailable, no increase in GUSB activity was detected in peripheral blood cells or in serum. However, vector derived sequence was detected in two of the dogs by PCR. For one dog there was a single positive time point two weeks after the transplant while the second dog remained positive for the duration of the study. Because of the difficulty in translating this technique from the mouse to the dog, the study was put on hold.

A vital aspect of the Society's funding was to help support the colony of MPS VII dogs and, in particular, those that had been successfully treated intravenously as neonates using a retrovirus vector. These dogs were 6 years old and had dramatic improvement in the skeletal, ocular, and

cardiac lesions but were at risk during a funding lapse from the NIH. The five treated dogs are now 7.5 years post-treatment and are being maintained to evaluate continued efficacy and possible long-term side effects of therapy. We presented a talk "Seven-Year Update for Neonatal Intravenous Retroviral Treatment of MPS VII Dogs" at the American Society for Gene Therapy in Boston. In addition, studies on MPS VII dog's bones and joints over the past year have resulted in two papers (Simonaro, C., D'Angelo, M., et. al. (2008) Mechanisms of glycosaminoglycan-mediated disease: Implications for the mucopolysaccharidoses and other connective tissue diseases. *Am J Pathol* 172:112-122, and Herati, R.S., Knox, V.W., et. al. (2008) Radiographic evaluation of bones and joints in mucopolysaccharidosis I and VII dogs after neonatal gene therapy. *Molec Genet Metab*, in press). We have also evaluated the effects of high and low serum GUSB activity resulting from retroviral gene therapy on the lesions in the brain and our preliminary conclusion, presented at the 10th International MPS and Related Disease Conference in Vancouver, is that high circulating enzyme appears to reduce GAG storage in the hippocampus.

In addition, we have now treated 10 MPS VII dogs intravenously as either neonates or at 45 days of age with adeno-associated viral (AAV) vectors of different serotypes. These dogs are not doing as well clinically as those treated with the retroviral vector, but are being maintained to evaluate longer-term therapy.

Future studies. 1) We have been successful in achieving prevention of many aspects of MPS VII using a retroviral vector. However, because of concerns about insertional mutagenesis and the risk of cancer, we have developed a self-inactivating retrovirus vector that should add a layer of safety to the treatment. Over the next year, we will make vector virus and administer it to MPS VII dogs to evaluate efficacy and long-term safety. 2) We will continue to evaluate the MPS VII dogs treated with AAV vectors. 3) We have now established a collaboration with the Penn Center for Musculoskeletal Disorders to evaluate the physical properties of ligaments, tendons, and articular cartilage in the joints of dogs with MPS VII. These data, combined with those of Lilla Simonaro and Kathy Ponder on the biochemistry of the structures, will be used to devise a pharmacological approach to therapy of the joints.

Dr Maria Fuller

Children, Youth and Women's Health Service

North Adelaide SA, Australia

"Membrane microdomains and improved clinical management for the mucopolysaccharidoses"

Received 9-08

The mucopolysaccharidoses (MPS) are characterised by the lysosomal accumulation of sugars, which is the primary cause of disease. However, the diverse and extensive array of clinical symptoms in MPS disorders suggest that many other cellular processes and functions are involved in the onset of symptoms and the rate at which they progress. One such process is likely to be the composition of certain fats and their distribution in cells. Within cells, specialised regions [or domains] of fat exist, which are known as rafts. Rafts are composed of certain types and structures of fats and they play important roles in cell communication and coordination; they are crucial for cell function. If the fat composition of the rafts is altered, it is likely that they cannot function properly and this may be a mechanism leading to disease in

MPS. With the funding provided by the National MPS Society, we hope to better understand the changes in these fats and attempt to correct these changes to see if we can bring them back to normal using conventional drugs and fatty acid supplementation. For the purpose of this study, we are using cultured MPS skin cells as cell models of the MPS disorders.

Aim 1: Determine the fat composition of specialised domains

In the laboratory environment, rafts can be isolated from other fats in the cell by their resistance to solubilisation by detergent, i.e. they float while the other fats are absorbed by detergent. However, in practice, isolating rafts from skin cells has proved to be problematic; the number of cells used in raft preparations and the growth rate have been shown to be important factors. Overcoming these particular problems has impeded our progress. We found that it was not possible to isolate rafts from slow growing cells and that 2 mg of cell protein was required. None of our unaffected control cell lines in the archives were suitable so we needed to collect fresh skin biopsies from healthy volunteers for this purpose. These cells are now growing well and raft isolation is underway. In the meantime, however, we have successfully isolated rafts from three MPS I cell lines and their fat composition has been determined by a sophisticated technique known as mass spectrometry. Once the rafts have been isolated from the control cell lines and have been fully analysed we will compare the differences between them and the MPS I cells. We will also then isolate rafts and determine the fat composition in MPS II, IIIA and VI cells.

Aim 2: Modulate the fat composition of the specialised domains back to normal

Even though we have not yet determined which fats are altered in MPS compared with unaffected controls, we have performed some preliminary experiments to demonstrate that we could alter the structure and types of fats within the MPS cells. Firstly, we inhibited the production of one of the fats by the addition of the drug myriocin to the culture media in which the skin cells are being grown. This was successful and reduced the amount of this fat in the cells by 50%. Next, to demonstrate that we can modify the structures of the fats in MPS cells we added fatty acids to the culture media. We showed that the addition of linoleic and oleic acids to the culture media was able to alter the structures of some of the fats present. Now that we have overcome the problems of raft isolation we will be able to complete Aim 1, and then we can identify which fats are altered and attempt to correct them in this aim.

Aim 3: Evaluate fats as biomarkers of disease in MPS

We have identified some potential fats that may be useful as biomarkers. Future work will involve measuring them in cells from MPS patients of different genotype and phenotype to evaluate their usefulness.

Dr. Synthia H. Mellon
Dept. of OB, GYN, Univ. of Calif. San Francisco
San Francisco, CA

“Neurosteroid treatment of MPS IIIA”?

Review not received; funding not provided for second year

2nd Year Research Reviews

Dr. Alessandra Biffi

“Novel efficacious and safe gene therapy approaches for the treatment of MPS I”

San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET)

Milano, Italy

received 8-09

The pipeline of the proposed project consisted in the comparison of the long-term efficacy in alleviating disease manifestations in the MPSI mouse model of three different therapeutic approaches:

- o Allogeneic hematopoietic stem cells transplantation (HSCT) from wild type donors (the treatment currently available for Hurler patients at disease onset);
- o Hematopoietic stem cell (HSC) gene therapy using a lentiviral vector (LV) encoding the human IDUA enzyme;
- o Liver-directed gene therapy using LV encoding the functional IDUA enzyme with the addition of miRNA sequences de-targeting expression from antigen presenting cells (APCs).

To this aim, during the 2nd year of funding we focused on:

1. Feasibility and safety of LV-mediated IDUA over-expression in human HSC

We generated a new strain of immunodeficient MPS1 mice, which were crossed to immunodeficient Rag2^{-/-} gamma chain ^{-/-} mice. The novel strain reproduces the main pathologic features of MPS1 and retains the key properties of Rag2^{-/-} gamma chain ^{-/-} mice. Indeed, these animals allow transplantation, engraftment and differentiation of human HSC. Therefore, they are currently been employed for assessing the therapeutic potential of human HSC (either from normal donors or MPS1 patients, upon gene correction with IDUA-encoding LV) in correcting MPS1 disease manifestations.

2. Long term efficacy and safety of HSC gene therapy in MPS1 mice (and comparison with WT HSC transplantation)

The efficacy of HSC-based gene therapy has been evaluated tested in IDUA KO mice (C57Bl6 background), which were transplanted at 2 months of age with either wipe HSC (transduced with GFP-encoding LV) or HSC from MPS1 donors, transduced with LV encoding the human IDUA. At the age of 8 months, reconstitution of enzymatic activity up to 100 fold above the levels detected in wild type mice was observed in peripheral blood mononuclear cells of mice receiving the gene corrected cells, whereas restoration of normal IDUA expression levels was seen in mice receiving the wild type HSC. At this time-point the phenotype of treated mice was evaluated by the means of functional, biochemical and histopathological studies. Wild type HSC transplantation allowed reaching physiological values of enzyme activity in the liver, spleen and kidney, but was unable to reconstitute detectable levels of IDUA activity in the hearth and brain of treated animals. Importantly, gene therapy led to enzyme over-expression in liver and spleen (to comparable levels with the hematopoietic system), and to reconstitution of enzyme activity in the kidney, hearth and brain at least up to the levels detected in the corresponding organs from wild type mice. Moreover, a rescue of major phenotypic abnormalities was observed in gene therapy treated mice as compared to controls. Gene therapy

treated animals showed amelioration of both the skeletal abnormalities and the behavioral performances as compared not only to mock-treated MPS1 controls, but also to MPS1 mice receiving wild type HSC. Histopathological evaluation of the tissues collected from all the experimental groups is currently on going.

3. Liver-directed gene therapy using LV encoding the functional IDUA enzyme with the addition of miRNA sequences de-targeting expression from antigen presenting cells (APCs) Due to preliminary data showing an apparent poor ability of the miRNA strategy to control immune responses in this setting, and to the clinical relevance of the results obtained with the HSC-based approach, we decided to re-focus on the latter in the perspective of generating data for a clinical development plan.

We would like to thank you for this funding opportunity and look forward collaborating with you again.

Dr. Mark Haskins
School of Veterinary Medicine, University of Pennsylvania
Philadelphia, PA

“Lentiviral Vector Therapy for MPS VII”?

Received 8-09

As reported last year, our initial experimental plan was based upon experiments in mice by our collaborator, Dr. Mark Sands, to use a lentiviral vector containing the human gene to treat MPS VII bone marrow cells in culture. In the previous year, we determined there were difficulties in translating this technique from the mouse to the dog and the study was put on hold.

A vital aspect of the Society’s funding was to help support the colony of MPS VII dogs and, in particular, those that had been successfully treated intravenously as neonates using a retrovirus vector. We have now had to euthanize two of the dogs, one with a hematoma of the spleen and one following an infection in her front leg. Both dogs had lived for ~8 years and were ambulatory, although with advancing age they and the other two dogs who are still alive in the colony find getting around more difficult. The dogs continued to have clear corneas and competent mitral valves.

In addition, we have now treated 10 MPS VII dogs intravenous as either neonates or at 45 days of age with adeno-associated viral (AAV) vectors of different serotypes. These dogs have not done as well clinically as those treated with the retroviral vector, but were maintained to evaluate longer-term therapy.

Future studies. 1) We have been successful in achieving prevention of many aspects of MPS VII using a retroviral vector. However, because of concerns about insertional mutagenesis and the risk of cancer, Dr. Kathy Ponder developed a self-inactivating retrovirus vector that should add a layer safety to the treatment. Over the next year, we will make vector virus and administer it to MPS VII dogs to evaluate efficacy and long-term safety. 2) We will continue to evaluate the MPS VII dogs treated with AAV vectors. 3) We have now established a collaboration with the Penn Center for Musculoskeletal Disorders to evaluate the physical properties of ligaments, tendons, and articular cartilage in the joints of dogs with MPS VII.

These data, combined with those of Drs. Lilla Simonaro and Kathy Ponder on the biochemistry of the structures, will be used to devise a pharmacological approach to therapy of the joints.

The funding by the MPS Society was critical to be able to keep the research on track during a lapse from the NIH. Thank you.

Dr. Calogera M. Simonaro

Mt. Sinai School of Medicine, New York, NY

“Pathogenesis and Treatment of the Mucopolysaccharidoses”

Received 7-09

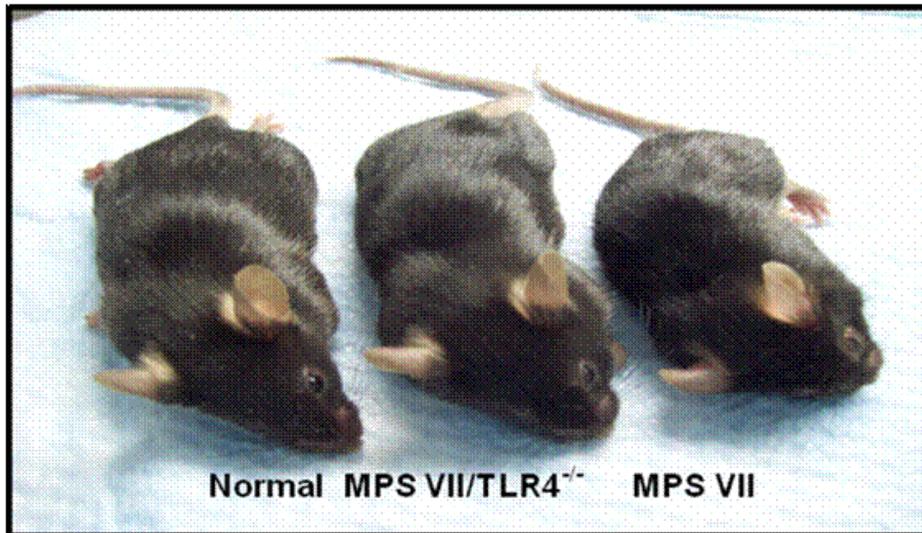
Therapies are available for some MPS disorders with limited effects in the bones and joints. Therefore, the overall goal of our research has been to use MPS animal models to study the disease mechanism in these tissues in order to develop new and improved therapeutic approaches. We have previously established that inflammation plays a major role in the pathology of MPS bones and joints, and that prevention of inflammation may have an important therapeutic effect. Our recent generation of an MPS mouse model with an inactivated inflammatory pathway (see below) has proven that inflammation has an important role in the pathogenesis of MPS bone disease. During the past year we have also continued long-term studies using the FDA-approved anti-inflammatory drug, Remicade™, in MPS VI rats. We are hopeful that completion of the Remicade™ studies in the MPS VI rats will provide a basis for the initiation of clinical trials, and “fast-track” approval of this (and perhaps other anti-inflammatory) drugs for MPS patients.

1. We have succeeded at generating MPS VII/TLR^{-/-} double mutant mice as the first “proof-of-principle” that the inflammation has a major impact on bone development. Consistent with our hypothesis and data, we have observed that inactivating TLR4 in the MPS VII animals has a remarkable effect on their growth and development. For example, inactivation of TLR4 in MPS mice led to significant increases in the length of the face and of the long bones. As illustrated by the figure on the attached page (Fig. 1), the double mutant MPS VII mice are much larger and have a more normal facial appearance than the MPS VII mice alone. Thus, in accordance with our hypothesis, activation of the TLR4 pathway in the MPS disorders is an important aspect of the disease pathogenesis, and inactivation of this pathway has a significant, positive effect. Continued funding from the MPS Society will allow us to continue to examine the biochemical, pathological and clinical changes in these double mutant mice.

2. Based on our previous findings, we have chosen to inhibit the downstream effects of TLR4 activation using the clinically available anti-inflammatory drug, Remicade™. This drug targets a molecule that is activated by the TLR4 pathway (i.e., TNF- α). Our results to date have shown that Remicade™ treatment can substantially reverse or prevent inflammation in the MPS VI rat model. We had previously shown that treatment of 6-month-old MPS VI rats with Remicade™ for 8 weeks reduced the levels of serum TNF- α to normal. We have now extended these findings and shown that a) in addition to TNF- α other inflammatory molecules also are reduced to normal in treated 6-month-old MPS VI animals, and b) inflammation can be prevented in MPS VI rats treated from 1 month of age for 24 weeks (as opposed to reversal in the 6-month-old animals; Fig. 2). Presently, studies are underway in the MPS animals to

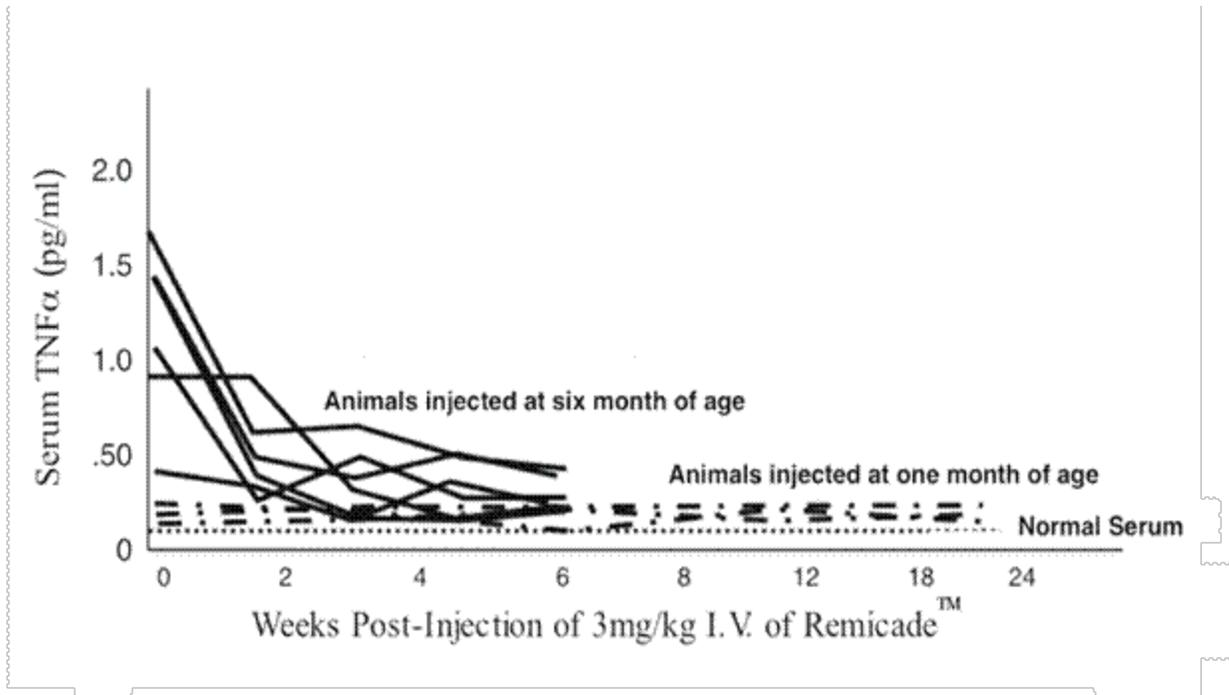
evaluate Remicade™ treatment in conjunction with Nalgazyme™ (ERT). We are confident that these studies will support our hypothesis that anti-inflammatory therapies may be important adjuncts for the treatment of the MPS disorders.

Figure 1.



The photo above is representative of a five-month old normal male, MPS VII/TLR4 and MPS VII mouse.

Figure 2.



Dr Maria Fuller

Children, Youth and Women's Health Service

North Adelaide SA, Australia

“Membrane microdomains and improved clinical management for the mucopolysaccharidoses”

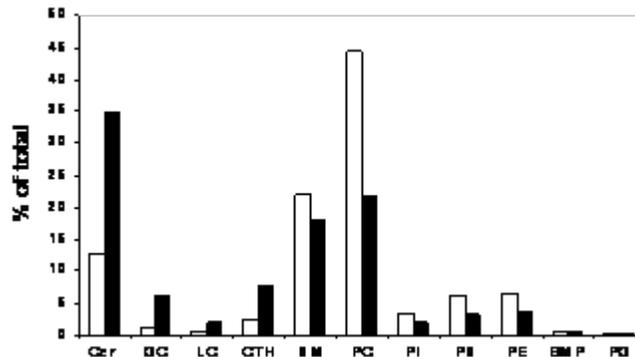
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The mucopolysaccharidoses (MPS) are characterised by the lysosomal accumulation of glycosaminoglycans, which is the primary cause of disease. However, the diverse and extensive array of clinical symptoms in MPS disorders suggest that many other cellular processes and functions are involved in the onset of symptoms and the rate at which they progress. One such process is likely to be the composition of certain lipids and their distribution in cells. Within cells, specialised regions [or membrane microdomains] of lipid exist, which are known as rafts. Rafts are composed of certain types and structures of lipids and they play important roles in cell communication and coordination; they are crucial for cell function. If the lipid composition of the rafts is altered, it is likely that they cannot function properly and this may be a mechanism leading to disease in MPS. With the funding provided by the National MPS Society, we have developed methodology to isolate rafts from cultured MPS skin cells (fibroblasts), which we are using as models of MPS disease. Furthermore, using a mass spectrometry “lipidomic” approach we have shown that the lipid composition of these raft domains in the MPS cells is different to that of the control cells.

In the first year of the project we developed methodology that would allow us to isolate lipid rafts from cultured skin fibroblasts reproducibly. We found that it was not possible to isolate rafts from slow growing cells and that 2 mg of cell protein was required. None of our unaffected control cell lines in the archives was suitable for this purpose so we needed to collect

fresh skin biopsies from healthy volunteers and establish new cultured fibroblast lines to be used as controls. Lipid rafts were then isolated from cultured skin fibroblasts from MPS patients and our healthy volunteers (controls) and the lipid composition was determined by mass spectrometry. In the raft domains we observed increases in the sphingolipid, ceramide and the glycosphingolipids, glucosylceramide, lactosylceramide and ceramide trihexoside, compared with our controls. Consequently, the lipid composition of the rafts was altered in MPS cells. In control cells, phosphatidylcholine was the major component comprising 45% of the lipid in the raft domain, whereas in the MPS cells, ceramide replaced phosphatidylcholine as the most abundant lipid comprising 35%, and reducing phosphatidylcholine to 20%. The Figure below shows the difference in the lipid composition between MPS I fibroblasts and controls. Additional changes in the raft composition included increases in the glycosphingolipids with concomitant decreases in the phospholipids. Of note was an alteration in the anionic phospholipid bis(monoacylglycero)phosphate which we have previously shown to be elevated in MPS (Meikle et al., 2008). Therefore, from our studies in cultured fibroblasts we have shown that the lipid composition of rafts is altered in MPS. It is unclear at this stage what effects an altered composition of lipid rafts has on cell function and how it may lead to disease.

Figure: Lipid composition of rafts



Lipid rafts were isolated from control skin fibroblasts (open bars) and MPS I fibroblasts (filled bars) and the lipid composition was analysed by mass spectrometry. The total amount of each lipid present in the rafts was expressed as a percentage of the total amount of all the lipids analysed. Cer, ceramide; GC, glucosylceramide; LC, lactosylceramide; CTH, ceramide trihexoside; SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; BMP, bis(monoacylglycero)phosphate; PG, phosphatidylglycerol.

To mimic what effect enzyme replacement therapy may have on returning the lipid raft composition back to normal, the culture media of MPS I fibroblasts was supplemented with recombinant human α -L-iduronidase. Following 24 hr of treatment with the enzyme, the glycosphingolipids were starting to normalize. Further studies are underway with increased time and concentration of α -L-iduronidase to see if these glycosphingolipids can be completely normalized. Importantly, treatment with enzyme showed no reduction in ceramide or bis(monoacylglycero)phosphate. In unaffected cells we have shown that myriocin added to the

culture media does reduce the amount of ceramide and further work is underway to test this in MPS cells. Additionally, lipid rafts are potentially modifiable by diet, particularly by dietary polyunsaturated fatty acids. Using control cells our initial results showed that supplementing the culture media with linoleic acid had no effect on the sphingolipids but did alter bis(monoacylglycero)phosphate. Further studies are underway to determine what effect linoleic acid has on MPS cultured skin fibroblasts, and whether combinations of drugs and fatty acids restore raft composition.

We are currently writing up our findings for publication and will send a copy of the manuscript to the society. We would like to thank the society for supporting our research and we hope to gain further independent funding to continue this work.

Meikle, P.J., Duplock, S., Blacklock, D., Whitfield, P.D., Macintosh, G., Hopwood, J.J. and Fuller M. (2008) Effect of lysosomal storage on bis(monoacylglycero)phosphate. *Biochem. J.* 411, 71-78.