

2011 Research Grants

The National MPS Society awarded \$421,500 in grant funding for 2011. The funding the Society provides has been and continues to be crucial as we move forward with our mission to find the cures.

We received 33 letters of intent from researchers around the world for the five grants offered in 2011. After reviewing those letters, our Scientific Advisory Board review committee requested full grant proposals from 13 researchers.

All grant recipients were awarded \$70,000 for the two year grant, with half of the total provided each year. The Society will fund \$25,000 to support the Lysosomal Disease Networks NIH grant research goals. The funding is designed for the Neuroimaging Core, which will benefit the four MPS projects. The Society also provides funding for post-doctoral fellows to attend scientific meetings, such as the American Society of Gene and Cell Therapy and the Gordon Conference on Lysosomal Diseases.

MPS II Grant

Vito Ferro, Small molecule chaperons for ERT for MPS II

MPS II is caused by defects in an enzyme called iduronate-2-sulfatase. Many of these defects result in degradation of the enzyme in cells before it has had a chance to carry out its normal function, thus producing clinical symptoms. MPS II patients may be treated with enzyme replacement therapy in which a synthetic, fully functional enzyme is administered by injection. Unfortunately, the replacement enzyme cannot cross the blood-brain barrier and thus cannot relieve the neurological symptoms associated with severe cases of MPS II. The aims of this project are to develop small molecules for the treatment of MPS II, which unlike enzymes, are capable of crossing the blood-brain barrier and thus may offer relief of neurological symptoms. The small molecules are designed to act as chaperones to protect the defective enzyme from degradation and restore enzyme activity to sufficient levels to relieve symptoms. This approach has shown great promise in other lysosomal storage diseases but has yet to be extended to MPS II. This project will address that situation.

MPS III Grant

Patricia Dickson, Choroid plexus-directed gene therapy as a source of intraventricular NAGLU-IGF2 for MPS IIIB

Animal models of MPS types I, II and IIIA can be treated by providing recombinant enzyme into the fluid surrounding the brain (cerebrospinal fluid). The application of this treatment of IIIB has been hampered by the inability of the missing enzyme, alpha-N-acetylglucosaminidase (NAGLU), to enter cells efficiently. We have created NAGLU tagged with insulin-like growth factor 2 (IGF2) which enters cells effectively using the mannose 6-phosphate receptor. Here, we will deliver NAGLU-IGF2 to the cerebrospinal fluid by using gene therapy in animal models. We will target the part of the brain that makes cerebrospinal fluid (the choroid plexus), to

determine whether this will provide a source of NAGLU-IGF2 for the brain. This study will provide proof-of-principle for choroid-plexus directed gene therapy with NAGLU-IGF2 as a potential therapy for MPS III IIB, to determine whether this approach can deliver enzyme using cerebrospinal fluid without the need for repeated injections.

General Grants

Alberto Auricchio, Gene therapy of MPS VI

Enzyme replacement therapy for MPS VI requires weekly administrations of costly enzyme and has a poor outcome on some of the disease characteristics including bone and cartilage abnormalities. We have recently shown that a single systemic delivery of an adeno-associated viral vector (AAV) encoding the correct copy of the enzyme missing in MPSVI results in:

- i. sustained expression of the enzyme from liver of MPS VI cats transduced by AAV;
- ii. significant amelioration of the disease phenotype (including bone and cartilage) in this large model of the disease.

Based on these promising results we are planning a clinical trial to test the safety and efficacy of our approach in MPS VI patients. Towards this goal we propose to:

- complete some of the pre-clinical data required for further clinical development
- develop bioengineered enzyme molecules with improved secretion or bone uptake which may increase the efficacy of gene therapy and lower the vector doses used in patients.

We believe these data will be instrumental to rapidly move gene therapy for MPS VI from bench to bedside thus overcoming some of the limitations of current therapies. In addition, the results from these studies may improve the cures for other MPS.

Adriana Montano, Role of inflammation in pathogenesis of MPS IVA

Morquio A disease is characterized with the build-up of two specific sugars (chondroitin-6-sulfate and keratan sulfate) in all the body cells, particularly in skeletal tissue. Effects of this build-up on the immune system and the consequences on the cartilage destruction and alterations of bone metabolism in Morquio A disease have not been investigated yet. We will clarify the role of immune system in the pathogenesis of Morquio A disease. We will characterize the immune profile of Morquio A mouse bone and cartilage cells and tissues, as well as Morquio A human cartilage cells. The outcome of this research will enable us to develop better approaches for treatment strategies to stop cartilage degeneration not only in Morquio A but also in other MPS.

Richard Steet, Blockade of cathepsin activity and TGF-beta signaling as a therapeutic approach for LSDs

Understanding the molecular events that cause disease symptoms is an important step in the development of new therapies for many inherited disorders, especially in cases where replacement of the defective gene or enzyme is difficult. In earlier studies, we showed that the

cartilage defects in a zebrafish model for ML-II are associated with increased activity of protein-degrading enzymes called cathepsins and excessive TGF-beta signaling. Our most recent work now demonstrates that reducing the activity of one of these enzymes, cathepsin K, results in correction of the cartilage defects in ML-II zebrafish embryos. Using known drugs, we now propose to block the activity of two other cathepsin proteases and to reduce excessive TGF-beta signaling to determine how these molecules impact the onset and progression of disease phenotypes such as impaired development of cartilage and heart valves. Since elevated cathepsin activity is a common feature of many MPS disorders, we believe our results on ML-II will increase our understanding of the disease mechanisms associated with other lysosomal diseases.

2011 1st Year Reviews

MPS II

Prof Vito Ferro

School of Chemistry & Molecular Biosciences, the University of Queensland

“Small molecule chaperones for EET for MPS II”

The aim of this project is the development of small molecule drugs for MPS II. Conventional small molecule drugs can be taken orally as a pill and have the potential to reach the brain in order to treat the more severe forms of MPS II, unlike enzyme replacement therapy (Elaprase) which can't cross the “blood-brain barrier”. Our approach is to develop compounds for so-called Enzyme Enhancement Therapy (or EET; aka “Pharmacological Chaperone Therapy”). This is an approach to treatment that has shown great promise in other lysosomal storage disorders, e.g., Gaucher's and Fabry disease, but has yet to be tried for the mucopolysaccharidoses such as MPS II. EET works by having a small molecule drug (a “chaperone”) attach itself to the defective enzyme, in this case iduronate sulfatase, and stabilizing it so it can do its intended job: to degrade the mucopolysaccharides in the cell. In order to prepare compounds for EET that are suitable for testing we need to synthesize small molecules that resemble the sugar iduronic acid, the component of the mucopolysaccharides that is degraded by iduronate sulfatase. The first step of this process is to prepare iduronic acid itself and then to make some chemical modifications to it. Iduronic acid is quite a complex sugar and is not commercially available, so in the first year of this project we have focused on developing methods of preparing iduronic acid from cheap and readily available glucose. We have investigated three different methods and one of them has so far shown the most promise. We have been able to prepare an important derivative of iduronic acid with modifications in the desired parts of the molecule. Our next aim is to transform this key intermediate into a range of compounds for testing as chaperones for iduronate sulfatase.

MPS III

Drs. Patricia Dickson and Stephen Kaler

UCLA Harbor, Los Angeles, CA

Choroid plexus-directed gene therapy as a source of intrathecal NAGLU-IGF2 for Sanfilippo B syndrome

The goal of this project is to study whether the choroid plexus could be made to produce NAGLU-IGF2 into the ventricles of the brain, and whether this will improve brain lysosomal storage in Sanfilippo B syndrome mice. The choroid plexus is responsible for production of cerebrospinal fluid, and if it could be made to produce a therapeutic enzyme, it would serve as a potentially permanent source of that enzyme for the brain. We produced a construct containing

the gene encoding NAGLU (the enzyme that is deficient in Sanfilippo B syndrome) fused to IGF2 (insulin-like growth factor 2). Manufactured forms of NAGLU lack mannose 6-phosphorylation, limiting their uptake into cells. To circumvent this problem, we attached IGF2 to NAGLU. IGF2 binds the mannose 6-phosphate receptor so that it can get NAGLU into cells without mannose 6-phosphate. Our studies in cells showed that IGF2 greatly improves intracellular uptake of NAGLU. This project began on July 1, 2011. Year 1 milestones achieved include: 1) re-establishment of the Sanfilippo B mouse colony in our laboratory, 2) production of adeno-associated viral vectors containing NAGLU-IGF2, 3) verification that the vectors produce intact, active NAGLU-IGF2 enzyme and 4) injection of adeno-associated vectors into the brain of normal rats. In year 2, we plan to complete the study the distribution of NAGLU-IGF2 in the brain of normal rats, select an effective dose, and perform a study to evaluate its distribution and effectiveness in Sanfilippo B mice. These experiments will provide proof-of-concept for choroid-plexus directed gene therapy for Sanfilippo B syndrome using NAGLU-IGF2.

General Grants

Alberto Auricchio (review will be available September, 2012)

Fondazione Telethon, Naples, Italy

Gene therapy of MPS VI

Adriana M. Montaña

Saint Louis University

Role of inflammation in pathogenesis of MPS IVA

Morquio A disease (Mucopolysaccharidosis IVA, MPS IVA) is an autosomal recessive disorder, caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). Patients with Morquio A disease have accumulation of the glycosaminoglycans, keratan sulfate and chondroitin-6-sulfate, mainly in bone and cartilage, causing systemic skeletal dysplasia. The broad goals of this research are to characterize the immune profile of Morquio A mouse model and to elucidate the role of cartilage and bone inflammatory reactions in the pathogenesis of Morquio A disease through investigation of secreted inflammatory factors involved in cartilage destruction and bone remodeling.

1. Characterization of the immune profile of the Morquio A mouse model.

Immune response to enzyme replacement therapy (ERT) is the principal limitation in the effectiveness of the treatment. The first step in the characterization of the immune profile of the knock-out Morquio A mouse model is to investigate the immune response after ERT.

We have found that Morquio A mice undergoing ERT have: i) the highest immune humoral response towards the recombinant human GALNS enzyme at 14 weeks of treatment, and ii) the highest cellular response at 16 weeks of treatment. This is consistent with previous observations where age of the mice and length of treatment play a role in the levels of immune response.

2. Characterization of expression profiles of genes associated to the pathogenesis of Morquio A disease.

We compared differences in inflammation profile of cartilage between Morquio A and wild type mice. We have found that there is up-regulation of several genes which play important roles in autophagy and apoptosis. We are in the process of quantifying and comparing these results at various ages in cartilage and bone cells of Morquio A and wild type mice.

Richard Steet, Ph.D.

University of Georgia, Athens, GA

Blockade of cathepsin activity and TGF-beta signaling as a therapeutic approach for LSDs

Investigating the molecular pathogenesis of lysosomal diseases such as the MPS and MPS-related disorders is a promising avenue towards the development of new therapies and can aid our understanding of the mechanisms that contribute to the onset and progression of disease symptoms. Over the past several years, we have taken advantage of a zebrafish ML-II model to investigate the pathogenic mechanisms that underlie the cartilage defects associated with this disease. We have identified and confirmed several target proteins (including cathepsin proteases and matrix metalloproteinases or MMPs) that exhibit increased and sustained activity in ML-II zebrafish embryos and hypothesize that inhibition of this excessive protease activity would result in therapeutic correction of ML-II associated phenotypes. In support of this hypothesis, we have demonstrated that inhibition of cathepsin K by genetic and pharmacological means leads to substantial correction of the cartilage defects in ML-II embryos as well as a surprising reduction in the activity of other proteases.

Over the past year, we have focused our efforts on determining whether other proteases such as cathepsin L and MMPs contribute to the disease process. Our results demonstrate that cathepsin L is subject to the same sustained activation in ML-II zebrafish embryos that we previously observed for cathepsin K (Petrey et al, *Disease Mechanisms and Models*, 5(2) 177-90 (2012)). These results are significant since they point to a common mechanism whereby this class of proteases is abnormally activated from immature forms. We believe this activation arises from the hypersecretion of these enzymes into the extracellular space upon loss of mannose 6-phosphate dependent lysosomal targeting. This hypothesis is supported by 1) the observation that levels of the mannose 6-phosphate recognition marker are greatly reduced or absent on the highly active, mature forms of cathepsins K and L in ML-II embryos and, 2) the direct visualization of cathepsin K exclusively within the extracellular space of developing cartilage of ML-II (but not control) zebrafish. Our near-term goals include an assessment of whether cathepsin L inhibition is capable of producing the same therapeutic benefit in cartilage as we noted with cathepsin K suppression. We have also obtained a specific inhibitor to MMP-13, an enzyme whose activity is increased in ML-II zebrafish. We intend to treat our ML-II model with this inhibitor and determine whether any of the phenotypes can be rescued or improved when this protease activity is decreased. Initial results indicate that this inhibitor can act on zebrafish MMP. Lastly, we have begun to manipulate the TGF-beta signaling pathway in ML-II zebrafish embryos to determine how altered regulation of this pathway relates to the disease phenotypes.

Since increased cathepsin and MMP activity, and dysregulation of the TGF-beta signaling pathway are common features of many MPS disorders, we believe our results on ML-II will inform our general understanding of the pathogenesis of other LSDs. Furthermore, our findings indicate that abnormal protease activation (in addition to increased expression) is an important

factor that should be considered in assessing the pathogenesis of these diseases. Finally, the demonstration that a reduction in cathepsin activity can provide some therapeutic benefit suggests that further investigation into small molecule protease inhibitors for the treatment of MPS and MPS-related disorders is warranted. We thank the MPS Society for their continued support of this research.

2011 2nd Year Reviews

MPS III

Drs. Patricia Dickson and Stephen Kaler

UCLA Harbor, Los Angeles, CA

Choroid plexus-directed viral gene therapy as a source of cerebrospinal fluid NAGLU-IGF2 for Sanfilippo B syndrome

The goal of this project is to evaluate whether adeno-associated virus (AAV) gene therapy vectors can remodel choroid plexus epithelia to produce N-acetylglucosaminidase (NAGLU) fused to insulin-like growth factor 2 (IGF2). The therapeutic purpose underlying our experiments is to enable secretion of the missing lysosomal enzyme into the ventricles of the brain in a mouse model of Sanfilippo B syndrome. Since choroid plexus epithelia turn over at an extremely slow rate, viral transduction of these cells with a correct version of NAGLU-IGF2 would potentially provide a permanent source of the enzyme in the cerebrospinal fluid for global brain distribution.

We produced a cDNA construct containing the gene that encodes NAGLU (the enzyme deficient in Sanfilippo B syndrome) fused to IGF2. Manufactured forms of NAGLU lack mannose 6-phosphorylation, which limits cellular uptake (Fig. 1). In contrast, NAGLU-IGF2 binds to the mannose 6-phosphate receptor so that delivery is greatly enhanced (Fig. 1). We documented robust expression and NAGLU enzyme activity (1.7 units/mg protein) in HEK293T cells transfected with the NAGLU-IGF2 construct. We next generated a recombinant AAV serotype 5 vector harboring NAGLU-IGF2 (rAAV5.NAGLU-IGF2) under the control of a chicken beta actin promoter and cytomegalovirus enhancer. The rAAV5 serotype is known from our previous work to target choroid plexus epithelia (Donsante et al., 2011), and we confirmed this in wild type neonatal mice (Fig. 2a). We then administered 5×10^{10} vector genomes of rAAV5.NAGLU-IGF2 to the left lateral brain ventricle of 12 week old Sanfilippo B mice (Naglu^{-/-}). Correct ventricular localization technique was confirmed in a subset of mice by dye injection. NAGLU enzymatic activity in brain sections reached several fold-normal (Fig. 2b) and immunohistochemical evaluation detected NAGLU-IGF2 throughout the brain and into neurons (Fig. 2c).

Our ongoing testing is evaluating the safety and efficacy of this approach to prevent or correct brain pathology and neurological abnormalities in older Sanfilippo B mice. This unique therapeutic approach combines the benefits of M6P-independent endocytosis and viral gene therapy to enable efficient NAGLU-IGF2 distribution throughout central nervous system. Our exciting preliminary results were accepted for presentation at the American Society for Gene and Cell Therapy annual meeting (May 2013, Salt Lake City, UT). The potential impact on clinical practice in the field of LSD is high since, if the proposed aims are successfully achieved, the

largest current barriers to health for patients with LSDs will be circumvented. In addition, the principles of gene transfer and CSF protein transport being investigated in this project will potentially be useful for other neurometabolic diseases with global effects on brain.

MPS II

Prof Vito Ferro

School of Chemistry & Molecular Biosciences, the University of Queensland

Small molecule chaperones for EET for MPS II

Final Report: National MPS Society Research Initiative 2011-2013 (extension to 2014)

The aim of this project is the development of small molecule drugs for MPS II. Conventional small molecule drugs can be taken orally as a pill and have the potential to reach the brain in order to treat the more severe forms of MPS II, unlike enzyme replacement therapy (Elaprase) which can't cross the "blood-brain barrier". Our approach is to develop compounds for so-called Enzyme Enhancement Therapy (EET; aka "Pharmacological Chaperone Therapy"). This is an approach to treatment that has shown great promise in other lysosomal storage disorders, e.g., Gaucher's and Fabry disease, but has yet to be tried for the mucopolysaccharidoses such as MPS II. This is thus the first time that this promising approach has been attempted for MPS II. EET works by having a small molecule drug (a "chaperone") attach itself to the defective enzyme, in this case iduronate sulfatase, and stabilizing it so it can do its intended job: to degrade the mucopolysaccharides in the cell. In order to prepare compounds for EET that are suitable for testing we need to synthesize small molecules that resemble the sugar iduronic acid, the component of the mucopolysaccharides that is degraded by the enzyme iduronate sulfatase. Two approaches have been explored for this purpose: (i) the synthesis of modified iduronic acid derivatives, and (ii) the synthesis of a compound known to bind to iduronate sulfatase, and derivatives of this compound. The initial stages of this process involved developing chemistries to prepare the required compounds. The first set of 8 test compounds are now in hand and preparations are in progress for their testing using purified enzyme and cell preparations from MPS II patients. The testing will be conducted by our collaborators at the Lysosomal Diseases Research Unit (LDRU) in Adelaide. Our collaborators have established a fluorometric assay for determining the affinity of the compounds for the enzyme. In addition, they have identified MPS II patient mutations that are likely to respond to chaperone therapy. Skin fibroblast cells from these patients will be used to test the compounds for chaperone activity, once Human Ethics Committee approval has been obtained. These studies should demonstrate cellular uptake of chaperone molecules and a reduction of cellular mucopolysaccharide levels, and should identify which mutations respond (best) to chaperone therapy.

We anticipate that the biological testing will identify the most promising candidate chaperones for further optimization and provide proof of concept that this is a viable approach for MPS II treatment. This project continues under funding from an MPS Society Grant (for 2013-2015) with the aim of generating sufficient preliminary data to obtain more significant research funding to accelerate this program towards clinical candidates.

General Grants

Alberto Auricchio

Fondazione Telethon, Naples, Italy

Gene therapy of MPS VI

Adriana M. Montaña

Saint Louis University

Role of inflammation in pathogenesis of MPS IVA

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To ameliorate both humoral and cellular responses in Morquio A mice we are looking at oral tolerance as an alternative. This will not only decrease the need of administration of immune suppressors to avoid immune responses but also will improve the efficacy of enzyme by avoiding presence of neutralizing antibodies to the enzyme used in ERT.

2. Characterization of expression profiles of genes associated to the pathogenesis of Morquio A disease.

The current study sought to understand if and how inflammation impacts the autophagic pathway in cartilage tissue of a Morquio A mouse model.

We performed expression analysis of over 60 genes using qRT-PCR in Morquio A and wild type mice at 1, 3,

9 and 12 months of age. We have found that inflammatory, apoptotic and autophagic markers were up-regulated in 12-month-old MKC mice compared to age-matched wild-type controls and compared to younger mice. These findings suggest that inflammatory receptors modulate autophagy and apoptosis in cartilage tissue of Morquio A mice. These data

could be used in developing future treatment modalities for bone growth abnormalities in Morquio A patients.

Richard Steet, Ph.D.

University of Georgia, Athens, GA

Blockade of cathepsin activity and TGF-beta signaling as a therapeutic approach for LSDs

We continue to take advantage of our ML-II zebrafish model to explore the relevance of cathepsin and matrix metalloproteases in both the cartilage and cardiac pathogenesis of this disease. Our published work in this area has demonstrated that inhibition of cathepsin K by genetic and pharmacological means leads to substantial correction of the cartilage defects in ML-II embryos as well as an unexpected reduction in the activity of other proteases. Using multiple approaches, we have also shown that this protease is hypersecreted from zebrafish chondrocytes and subject to abnormal proteolytic activation to its mature form.

Over the last year, we have begun to assess the role of other proteases (cathepsin L and MMP13) in the cartilage phenotypes. Using a specific inhibitor to MMP-13 (whose activity is increased 10-12-fold in ML-II zebrafish), we showed that a maximal inhibition of 70% could be achieved in embryo lysates. Despite this level of inhibition, no phenotypic correction of the cartilage phenotype was observed in the embryo, suggesting that this protease is not a central contributor to the developmental defects in this particular tissue. MMP13's contribution to later stages of disease and to other tissues (such as the cardiac defect) warrants further exploration. More recently, we have begun investigating the pathogenic contribution of cathepsin L and have shown that this protease also appears to undergo abnormal proteolytic activation in ML-II embryos. Since four homologous cathepsin L genes are now known to exist in zebrafish, we are currently characterizing the expression and localization of their individual transcripts. These analyses, which serve as a prelude to genetically manipulating cathepsin L expression, have revealed that one previously unidentified gene, *ctsL1b*, is the only isoform with increased transcript levels in the ML-II model. This is important, as our original transcript analyses did not distinguish between the four isoforms. This is also consistent with the finding that morpholino-knock down of the cathepsin L1a isoform did not reduce total cathepsin L activity. In light of these new expression studies, we are continuing to manipulate cathepsin L expression, with *ctsL1b* as the main gene targeted.

Another goal of the proposed research was to address the relevance of altered TGFbeta signaling in ML-II pathogenesis. Investigation of this pathway and its role in various ML-II phenotypes is underway, and has led to the identification of several molecular hallmarks for both the cartilage and cardiac defects. We are now positioned to determine whether directly manipulating TGFbeta signaling impacts the phenotypes.

The demonstration that a reduction in cathepsin activity can provide some therapeutic benefit suggests that further study into small molecule protease inhibitors for the treatment of MPS and MPS-related disorders is warranted. We thank the MPS Society for their continued support of this research and remain hopeful that the avenues of research that stem from this work can be translated into therapies for MPS and MPS-related disorders.

