RESEARCH

Columbus Children's Research Institute - Dr. Haiyan Fu

We have been studying gene therapy for treating the neurological disorders of MPS IIIB, with the support of NIH, and the MPS community through Ben's Dream-The Sanfilippo Research Foundation and Children's Medical Research Foundation. We made an adeno-associated virus (AAV) vector (vehicle carrying genes into cells), containing the gene of normal human NaGlu, which is missing in MPS IIIB patients. One of the biggest challenges in therapeutic development for MPS IIIB and other lysosomal storage diseases, is how to deliver therapeutic materials efficiently into the central nervous system (CNS). We have developed two non-surgical procedures to deliver the AAV vector into the CNS of MPS IIIB mice, which resulted in significantly increased lifespan and improved behavioral performances. Our data suggest that our AAV gene therapy procedures greatly slowed down the progress of the CNS disease in MPS IIIB mice, though it did not effect a complete cure. The data gained in these studies showed the potential for using AAV gene therapy to treat MPS IIIB. Although we continue our research to improve the therapeutic efficacy of AAV gene therapy on MPS IIIB in animal model, we feel that we have a therapy that provides meaningful benefits in our MPS IIIB animal model. We have designed the animal therapeutic experiments so that they can be applied to human clinical therapies. We feel that the time has come to consider translating our AAV gene therapy procedure(s) from mouse studies into human clinical application. We recently submitted an application to NIH for a translational research grant, which may support us to go through the procedures for an IND application from FDA for clinical trials using our AAV gene therapy approach in MPS IIIB patients, if we can get the grant.

We continue our studies, which, at this time, are focused exclusively on MPS IIIB, its pathology, and mechanisms of disease progression, in order to better understand the disease and further benefit therapeutic research, since MPS IIIB is much more complex than we used to know.

I appreciate that you expressed interest in helping to further our MPS IIIB research. While we continue to have financial support from the Ben's Dream-Sanfilippo Research Foundation, funds from the NIH and federal agencies have declined in recent years, and this has impacted our research program. Federal funds for rare diseases are particularly scarce. We would appreciate your help in any way, whether with fundraising, or with political support for additional funding for genetic diseases like MPS IIIB, that affect the central nervous system.

Haiyan Fu, PhD.
The Center for Gene Therapy
The Columbus Children's Research Institute
700 Children's Drive
Columbus, OH 43205
Tel. (614)355-3572
Fax. (614)722-3273
email: FuH@ccri.net

University of California, Los Angeles, Dr. Elizabeth Neufeld

The major obstacle to treatment of the Sanfilippo syndrome by enzyme replacement is the inability of therapeutic enzyme to get into the brain because of the blood-brain barrier (BBB). The BBB is a property of capillaries in the brain, which form an essentially impermeable barrier to large molecules such as enzymes. However, the capillaries make an exception for molecules that the brain requires. For example, the brain requires iron, and the capillaries have a mechanism for bringing it from the blood to the brain. Specifically, the iron is bound to transferrin (a protein that carries iron), which in turn binds to transferrin receptor molecules on the blood side of the capillaries, shuttles with the receptor across the cell and is released on the brain side. This mechanism of transport from one side of the cell to the other is called "transcytosis". Our strategy is to modify lysosomal enzymes so that they can bind to the transferrin receptor and be ferried by transcytosis across the capillary cells into the brain, where they could be taken up by neurons.

We have set out to modify lysosomal enzymes by "aptamers" - synthetic small molecules of nucleic acid (RNA or DNA). Aptamers that bind to any desired molecule can be isolated from very large random mixtures of chemically synthesized RNA or DNA. Thus, we have isolated aptamers that bind to the mouse transferrin receptor. We can attach these to model proteins (for example, streptavidin or albumin) and show that such molecules can be taken up by mouse cells in cell culture. Our next goal is to determine whether -N-acetylglucosaminidase, administered intravenously to MPSαaptamer-modified IIIB mice, can be delivered to the brain and correct the pathology that is specific to the disease. This study is funded in part by the National Institutes of Health and in part by the Children's Medical Research Foundation.

Work recently completed: characterization of some storage products in the brain of the MPS III B mouse.

In 1999, we had generated a mouse model of MPS IIIB by -N-acetylglucosaminidase.αdisrupting the Naglu gene, which encodes the enzyme We had found that heparan sulfate, a mucopolysaccharide which cannot be degraded for lack of the enzyme, was stored in excess in many tissues of the MPS IIIB mouse, but rather surprisingly, relatively little heparan sulfate was stored in the brain. We have now found that storage of heparan sulfate was greatest in specific areas of the brain. The areas storing heparan sulfate also accumulated -N-acetylglucosaminidase for degradation. This material that does not require other material includes the lipids cholesterol and GM3 ganglioside, and the proteins ubiquitin and SCMAS. Storage of these substances starts when the animals are very young and increases as they age. It is not unique to MPS III B but occurs, to a greater or lesser extent, in many other lysosomal storage diseases. Our interpretation is that the primary storage material (heparan sulfate in the case of MPS III B) causes lysosomes to become greatly enlarged and more numerous, which in turn must generate many signals to which the cell responds, by unknown pathways, with secondary accumulation of these other substances. We don't know how these secondary accumulations affect brain function, but we can use them as "markers" when studying the progression of the disease and the effect of therapy in animal models.

The National Institutes of Health and the Children’s Medical Research Foundation funded this study.

University of North Carolina - Chapel Hill, Dr. Joseph Muenzer

Gene therapy research on Sanfilippo B Syndrome (MPS III B) directed by Dr. Joseph Muenzer has been made possible at the University of North Carolina at Chapel Hill (UNC) by the support of The Children's Medical Research Foundation, Inc. The goal of the MPS III B research at UNC is to develop methods using gene therapy to express or produce the enzyme missing (N-acetylglucosaminidase and abbreviated NaGlu) in MPS III B in the central nervous system. If the enzyme is made in adequate amounts and in the correct form, the storage of glycosaminoglycan (GAG) could be reversed or prevented from further occurring. This MPS III B gene therapy research at UNC has been possible because of a MPS III B animal model developed and provided to UNC by Dr. Elizabeth Neufeld and co-workers at UCLA.

Dr. Muenzer's laboratory has focused on using the adeno-associated viral (AAV) vectors as the method to deliver the human NaGlu gene into the brain of MPS III B animals. AAV vectors containing the coding sequence for the NaGlu gene have been made. Dr. Muenzer's laboratory has successfully produced the NaGlu enzyme using AAV gene therapy and corrected the GAG storage in vitro in MPS III B human fibroblasts. The NaGlu enzyme has also been expressed using AAV gene therapy in many brain areas of MPS III B animals. Studies are in progress to determine how best to produce the NaGlu enzyme in the central nervous system and to determine if the expressed NaGlu enzyme in the MPS III B animals is able to correct GAG storage. The storage of GAG in cells and tissues is due to the missing enzyme NaGlu which causes the clinical problems seen in children with MPS III B.

Although, we have not succeeded in proving that the enzyme produced after the injection of AAV vectors into the brain of animals corrects the GAG storage, studies with primary mouse brain cell cultures are very encouraging. We have been able to establish primary cultures of MPS III B mouse brain, kidney, liver and skin fibroblast cells. The AAV gene therapy vectors, when added to the cell cultures, are capable of producing the NaGlu enzyme in these MPS III B primary cell cultures, including brain cell, and the storage of GAG is corrected. In addition, AAV expressed enzyme is secreted by the cultured brain cells into the culture media. This secreted enzyme is also able to correct the storage of GAG when added to MPS III B mouse brain cells in culture. These experiments support the concept that AAV vectors can deliver the MPS III B human gene to brain cells, and the enzyme produced will correct the storage of GAG in the central nervous system.

The MPS III B research at the University of North Carolina in Dr. Muenzer's laboratory has been performed by Dr. Haiyan Fu in collaboration with Dr. Jude Samuski (Director, UNC Gene Therapy Center). The goal of Dr. Muenzer's laboratory is to develop the preclinical animal data demonstrating successful enzyme production and correction of storage in MPS III B prior to applying to the Food and Drug Administration for permission to submit a Phase I AAV gene therapy clinical trial for Sanfilippo B Syndrome.

University of Minnesota, Dr. Chester Whitley

Our laboratory is highly focused on developing gene therapy for MPS diseases, and with this grant from the Children's Medical Research Foundation, has made significant advances during the past two years. This grand funds the work of Dr. Hong Zhao, who was the first to identify and characterize the gene causing Sanfilippo B Syndrome, while working in the laboratory of Dr. Elizabeth Neufeld (UCLA). The specific aims and accomplishments of Dr. Zhao and her colleagues illustrate the progress of a research concept as it develops from the laboratory bench toward potential clinical application.

Based on previous experience with mutation analysis methods developed in the laboratory of Dr. Whitley, Drs. Zhao and Elena Aronovich developed a method of rapid automated sequencing of the human NaGlu (Sanfilippo B gene) coding region, which allowed identification of the mutations responsible for defective NaGlu enzyme. While manual sequencing of a gene of this size normally requires several months' work, the method developed by this group showed that it is possible to accomplish automated sequencing of a gene in as little as a week. Most importantly, this method provides the molecular genetic background to insert the normal gene sequence into a retrovirus vector, or other gene transfer vehicles, for subsequent investigations of therapeutic gene delivery and expression.

Initial gene therapy experiments would be to insert the NaGlu cDNA sequence into a virus-derived vector system and then study gene transfer into human blood cells. Investigators would then evaluate whether or not one or more such vectors will yield high levels of normal NaGlu enzyme. If so, such a vector could be used to provide a "super bone marrow transplant" which after modification of some of the currently used clinical approaches, could be used to transfer the normal gene into blood cells of affected individuals. The potential advantage would be the ability to provide high levels of enzyme in the blood, but without the problems and risks of "graft vs. host disease." Such vectors are currently in development.

Medical College of Georgia – Augusta, GA, Dr. Robert K. Yu

Mucopolysaccharidoses (MPS) are a group of inheritable genetic disorders. Owing to the defective degradation of naturally occurring substances, collectively called glycosaminoglycans (GAGs), these materials will accumulate in tissues causing disturbances in the normal physical and mental development of the body. The disease proceeds with severe neurological symptoms initiated in early childhood.

Among the various forms, MPS III (Sanfilippo Syndrome) is caused by deficiency in one of four degradative enzymes, leading to an accumulation of GAGs, such as heparan sulfate, to give rise to four different variants of the disease (A, B, C, and D). The accumulation of heparan sulfate is frequently accompanied by the accretion of gangliosides. These substances are important constituents of the nervous system and are known to play a crucial rose in normal brain development and function. The accumulation of abnormal amounts of these substances is known to contribute to the disease process. Recent studies from our laboratory have shown that the accumulation of ganglioside in Sanfilippo Syndrome is very likely the result of an impairment of enzymes involved in ganglioside metabolism as a result of the accumulation of heparan sulfate.

In recent years, enzyme replacement, bone marrow transplantation, and gene therapy have been proposed as therapeutic strategies for MPS and related lysosomal storage disorders. However, there are difficulties associated with these strategies for correcting the genetic defects in the brain because the brain is a well-protected organ. Most of the strategies mentioned above cannot be easily applied to the brain.

Since in a variety of gangliosidoes, the stored gangliosides are known to contribute to the development of mental retardation, it has been suggested that a reduction of the ganglioside content by enzyme replacement therapy, gene therapy, or metabolic control, may alleviate the mental symptoms of the disease. We already have tested the strategy of inhibiting ganglioside production under conditions of low toxicity to cells. This approach will be attempted in an animal model of Sanfilippo Syndrome established by Dr. Elizabeth Neufeld at UCLA. Several drugs that are active as inhibitors of ganglioside production are commercially available and will be orally or intravenously administered to the mouse. The efficacy of this therapeutic approach will be monitored by analysis of the ganglioside content in the nervous system, structural examination of the affected tissue, and behavioral testing of the treated mice.

In addition, the Sanfilippo mouse model also will be used for experimental analysis of two novel approaches for the treatment of the disease. In the first approach, cell replacement therapy, we will investigate the possibility of grafting normal glial cells (essential elements of nervous tissue) into the brain of Sanfilippo mice. We hope the grafted normal glial cells can correct the metabolic defects in abnormal nerve cells in these mice. This strategy has been successfully applied to the treatment of a number of neurodegenerative diseases, such as Multiple Sclerosis and Parkinson’s Disease. After we have tested the efficacy of this strategy in animals, we will test the possibility of using cell replacement therapy for the treatment of Sanfilippo Syndrome.

Another approach using the Sanfilippo mouse model is direct inhibition of enzymes involved in GAG production. Inhibitors of GAG production have been described in studies with cultivated cells. However, nothing is known about the potency of these inhibitors in animal models. We will initiate a detailed investigation using specific inhibitors of GAG production as well as other potential drugs for the effective treatment of Sanfilippo Syndrome.

In summary, we plan to employ the Sanfilippo mouse model for the development of a treatment strategy using three different approaches: 1. inhibition of ganglioside biosynthesis by application of commercially available enzyme inhibitors; 2. cell replacement therapy by transplantation of normal brain cells, and 3. inhibition of GAG biosynthesis by application of existing or chemically modified drugs.

It is anticipated that following the animal studies, we will proceed with clinical trails for patients with Sanfilippo Syndrome. The development of rational and effective therapeutic approaches should be of great interest to patients who are suffering from this debilitating disorder. Additionally, these strategies should prove applicable to other similar neurodegenerative diseases which affect many more children.

During the past year, we have completed a detailed study of the ganglioside compositional analysis of goat brain with Sanfilippo disease (MPS IIID). In addition, we also have finished a study to characterize the ganglioside composition of a mouse stem cell line, with the goal of using stem cells as a potential source for cell therapy. In addition to the testing of a drug therapy, which is on-going, we plan to develop this as an effective strategy for cell therapy, which promises to be more permanent. We also have established in our laboratory a Sanfilippo mouse colony provided by Dr. Elizabeth Neufeld, UCLA. These animals will be used for testing our proposed treatment plans using drugs and cell transplantation.