The Children's
Medical Research
Foundation, Inc.®

KirbyGram®

The latest news on Kirby Wilson and friends and the search for a cure for Sanfilippo Syndrome

June 2006

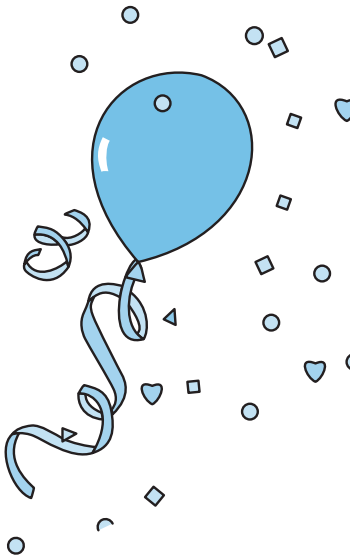
What is Sanfilippo Syndrome?

Sanfilippo Syndrome is one of seven Mucopolysaccharide (MPS) disorders. There are four different enzyme deficiencies that cause Sanfilippo. The Sanfilippo disorders are described as type A, B, C, or D. There is very little difference between the four types, though there have been a few very mild cases of the B form reported where the children have remained relatively healthy into early adult life.

Children with Sanfilippo are missing an essential enzyme that breaks down a complex body sugar called heparan sulfate. This sugar slowly builds in the brain, stopping normal development and causing hyperactivity, sleep disorders, loss of speech, dementia and typically, death before adulthood. There is no cure yet.

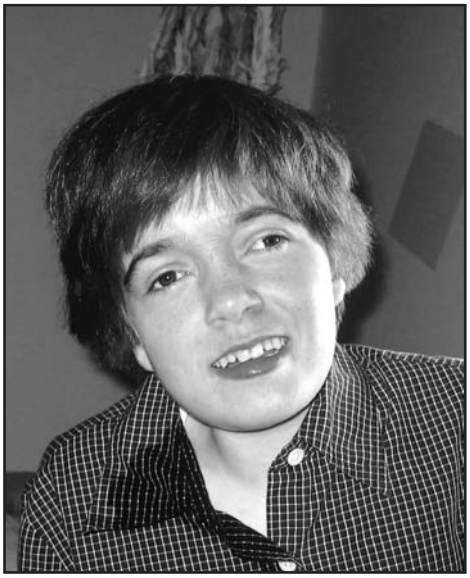
While Sanfilippo occurs once in 24,000 births, successful research into the disease could apply directly to many of 5,000 other genetic disorders.

KIRBY UPDATE



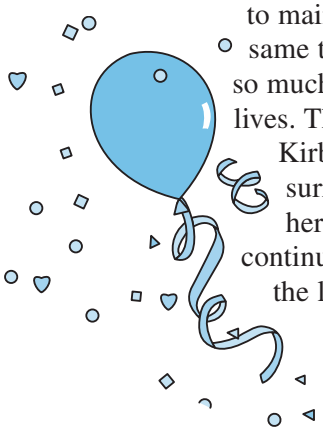
April 15th marked Kirby's 15th birthday! The warm, sunny day included everything that continues to bring happiness to Kirby's days -- outdoor play on her teeter-totter and swing, hot dogs and chips for lunch and, of course, pizza and Coke for dinner as her loving family and friends surrounded her.

Sue comments, "It is hard to believe that Kirby is 15 years old. Her strength and perseverance over the years have been incredible. As she now struggles to walk, there are days it seems, as parents, our worst fears are coming true. But then, there is that smile, the one that brings incredible joy to our hearts and does not allow us to dwell on our fears. I am reminded of something a father, who had just lost his son, recently wrote on his observation in the hospital of other parents with critically ill children. He stated it was a powerful experience, and he and his wife sensed "a collision of the parents' hopes and fears." I could not possibly describe Brad's and my emotions at this time any better."



Our Birthday Girl.

She continues, "It is an extraordinary helpless and fearful experience to watch your dear, tenacious bundle of joy struggle to maintain. But at the same time, there remains so much good in our lives. There are people like Kirby's school staff, with their dedication and compassion, who surround her each day and are so willing and able to challenge her to be the best she can be. There are the scientists who continue to make amazing strides toward bringing their work from the laboratory to human trial. There are the people who embrace our family with their love and support of our Foundation and its mission. And, there is Kirby, who is still fighting Sanfilippo with everything she has. It truly is a most powerful collision of our hopes and fears."



FUNDRAISING NEWS

The Foundation Gives Thanks…

To **Gresham, Smith and Partners**, of Nashville, TN, for their most generous support of the Foundation this past holiday season as they chose "To celebrate the season of giving" with a donation to the Foundation in honor of their business friends and associates.

To **Crowe Chizek and Company,** in Oak Brook, for its donation to support the Foundation and to **Rich Brummet**, honorary board member, who presented the Foundation to Crowe's Charity Committee for consideration.

To **Dr. S. J. Sanfilippo**, the doctor who first described the condition in 1963, for his kind words and support of the Foundation.

To **Gabriella Bucci,** this year's LaGrange Highlands Talent Show coordinator, who requested all thank you gifts to her be in the form of a donation to the Foundation.

To **Peter John** for his donation to the Foundation in fond memory of **Maureen Spinuzza**.

To **Fred and Beth Angeli** for their donation in memory of **Everett Frailing.**

To **White Hen Pantry, Kirschbaum's Bakery and Casey's Market**, all from Kirby's hometown of Western Springs, for their continuous fundraising efforts using cash jars. And to the people of the community who fill them, your "hometown girl" thanks you from the bottom of her little heart.

To our dear friends at **Highlands Presbyterian Church** for their continued love and support of our family and Foundation.

And To The Families Working Together For The Cure….

To **David and Anna Kidwell**, of Louisville, KY, for their most generous and steadfast support of the Foundation and its mission in honor of their daughters, **Brooke and Ashleigh**.

The Kidwell Girls: Callie, Brooke, Danielle and Ashleigh.

To **Arthur and Roberta Kidwell**, proud grandparents of **Brooke and Ashleigh**, for their donation.

To **Steve and Betsy Fowler**, of Shelby Twp., MI, for their donation in honor of their daughter, **Kimberly**.

To **Cheryl Deep and Lance Miller** for their donation to the Foundation in honor of **Sydney and Hunter Moff** of Williamsport, PA.

To **John and Joan Bellontine** for their efforts in planning a run/walk scheduled for June 17th, in honor of their daughter, Grace. A portion of the proceeds from this "race for a cure" will go to the Foundation.

To **Catherine Gladitsch** for requesting donations to the Foundation in honor of **Grace Bellontine**, in lieu of gifts in celebration of her 40th birthday.

To the **Hudson Emblem Club** of Hudson, MA, for its donation to the Foundation in fond memory of **Andrew Slattery**, of Ventura, CA, who passed away last June after battling Sanfilippo for almost 11 years.

Grace Bellontine

FUNDRAISING NEWS

News From Connecticut

The Foundation is extremely grateful to the people within the Logan's community who continue their support of "Rhianna's Hope," as the Logan's days remain centered around ensuring appropriate care and therapeutics for Rhianna.

Justin and Tabitha Manafort have helped to keep hope for a cure high for years with their most generous and steadfast donations to the Foundation and, most recently, made a donation to the Foundation in memory of their aunt, **Mary Fuschillo**.

A donation was received from **Robert Ibitz**, a car enthusiast, who in the past has used his passion for cars as a fundraiser for Rhianna.

The Artisan's Marketplace holiday fundraiser continues to grow in popularity. The "Santa for Hope" raffle and "Stars for Hope" made more than $1,126 for the Foundation. "Stars for Hope" is a craft kit for children put together right at the shop by Rhianna's family and her friends. Customers purchase the kit, allowing their children to create the star and keep it, reminding them of Rhianna. Or they can return the star to the Artisan's Marketplace, where others can purchase it, doubling the donation to the Foundation. What a shining example of compassion.

And, as always, our thanks to **Cory and Donna Gabel**, Rhianna's aunt and uncle, for their ongoing donations of a portion of the proceeds from Cory's solo piano instrumental CD, "One Road," to the Foundation. Sales continue, as do donations to the Foundation. Interested in this new "spin" to help? Just contact the Foundation to obtain a copy or log on to Cory's website at cdonavan.com.

Rhianna Logan taking a swim with friends.

RESEARCH UPDATE

Haiyan Fu, Ph.D.
Center for Gene Therapy, Columbus Children's Research Institute, Department of Pediatrics, College of Medicine, Ohio State University

Global neurological degeneration is the primary cause of high mortality and premature death in MPS IIIB (Sanfilippo B) patients. Therefore, one of the critical issues in developing therapies for MPS IIIB is to be able to deliver therapeutic reagents (including gene delivery vectors or enzymes) to the entire central nervous system (CNS), and not just to a localized brain area. The presence of the blood-brain barrier (BBB) prevents the IV injected vectors and enzymes from entering the CNS.

Our long-term goal is to develop gene therapy using adeno-associated viral (AAV) vector to treat MPS IIIB patients. To achieve this goal, our efforts have been focused on two major aspects. First, we must achieve widespread distribution of the recombinant AAV (rAAV) gene delivery vectors in the CNS. Second, we must maximize the therapeutic benefits of the recombinant gene delivered by the rAAV vector for the CNS disorders in the MPS IIIB mouse model.

In our previous studies, we have made rAAV vectors (means of carrying genes into cells) containing the gene of normal human a-N-acetylglucosaminidase (NaGlu), the enzyme missing in MPS IIIB patients. We have previously shown that our AAV vectors mediated the production of functional NaGlu and corrected the storage of glycosaminoglycan (GAG, previously called mucoploysaccharides) in cultured MPS IIIB cells. We also delivered the AAV vector into MPS IIIB mouse brain by direct injection, and demonstrated the long-term, but only localized, production of the missing enzyme and the localized correction of lysosomal storage in the brain area related to the injection sites.

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In our further efforts, we developed two non-surgical approaches to deliver rAAV vectors into the CNS of adult mice. We demonstrated a broad spread of rAAV vector in the CNS by an intracisternal (IC) injection. We also established an intravenous (IV) injection procedure following mannitol pretreatment to temporarily disrupt the BBB, and achieved a global distribution of AAV vector in mouse CNS. We believe that these vector delivery approaches will provide a solid foundation for gene therapy for MPS IIIB, as well as other types of MPS with CNS manifestations. We also anticipate that combining IV and IC injection may enhance the CNS delivery of rAAV vectors and provide therapy for other affected tissues and organs as well.

We have used these new approaches in our ongoing therapeutic studies over the last few years. We have treated young adult MPS IIIB mice with rAAV2-hNaGlu vector by an IV injection, an IC injection, or a combination of IV and IC injection. We pretreated the mice with an IV infusion of mannitol. After these treatments, we observed a decrease of pathological lysosomal storage in the brains of all the treated MPS IIIB mice, with the NaGlu activity reaching 10-100% of normal levels. The IV vector injection lead to the clearance of lysosomal storage in liver, and partial correction of storage in other somatic tissues. Most importantly, these treatments significantly prolonged the lifespan of MPS IIIB mice to 11.1-19.5 months (IV+IC injected), 9.2-15.9 months (IV injected), and 10.3-17.5 months (IC injected), compared to 7.9-11.0 months in non-treated MPS IIIB mice. Furthermore, we also observed improved behavioral performance in 72% of IV+IC treated MPS IIIB mice. These behavioral improvements included increased activity, normalized swimming speed, and nearly normalized performance in a visual cue task in a water maze, which indicates improvements in learning ability. These results demonstrated a positive therapeutic impact of IV+IC delivery of rAAV vector carrying the correct NaGlu gene for the treatment of the CNS disorders in MPS IIIB mice, though it did not effect a complete cure. We also wish to emphasize that we designed our therapeutic experiments in mice to fit the requirements, in terms of feasibility and scale, for human application.

Furthermore our recent experiments demonstrated that the optimal timing for IV injected rAAV vector to enter the CNS was 8 minutes after mannitol pretreatment. The number of transduced brain cells was increased approximately 10-fold when the vector was IV injected at 8 minutes after mannitol infusion, compared to that at 10 minutes after mannitol pretreatment, which was the timing we used in our previous therapeutic experiments, as described above. We anticipate that using this optimal timing will further improve the therapeutic impacts of our rAAV gene therapy procedures on MPS IIIB.

Our studies showed a great potential to treat MPS IIIB with rAAV gene delivery vectors. However, more effort is needed to achieve the desired therapeutic goals. Our ongoing research will be focused on improving the efficacy of rAAV gene therapy for the CNS disorders of MPS IIIB, by further optimizing our vector delivery strategies and enhancing the enzymatic functions of rAAV-expressed NaGlu. We are also working toward the future clinical application of AAV gene therapy for treating MPS IIIB patients.

Robert K. Yu, Ph.D., Med.Sc.D
Institute of Molecular Medicine and Genetics, Medical College of Georgia

Dr. Robert Yu and his team of scientists are using neural stem cells (NSCs) that can be transplanted into the brain to correct some of the metabolic defects in Sanfilippo disease (MPSIII). This is one of the genetic disorders collectively called mucopolysaccharidoses (MPS). In these disorders, there is an abnormal accumulation of heparin sulfate and/or condroitin sulfate in the body that may be harmful to normal health. Our effort is a part of a major undertaking to utilize stem cells for reducing the abnormal accumulation of these compounds and for achieving neural repair. Stem cells are self-renewable, capable of differentiating into many cell types, such as muscle,

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bone, and nerve, and have been shown to be capable of repairing damaged tissues and organs. Our goal is first to transplant NSCs into the brains of animal models with MPS to see if these cells can correct the storage of heparan sulfate by serving as a source of the corrective enzyme to other neighboring cells in the brain. Dr. Yu and his team are first studying the biological properties of NSCs in test tubes and in the brain of animals with MPS storage defects. This basic biological knowledge is essential because they want to make sure that they can produce sufficient numbers of these cells for transplantation. After the stem cells are transplanted, they want to make sure that the transplanted cells survive, migrate, and grow normally within the recipient's brain. In other words, they want to note if there is any abnormal (i.e., tumorous) growth of these cells in the brain. Dr. Yu plans to determine which of these stem cells serve best in transplantation therapy. After he has obtained the most desirous cells, he will transplant them into animals with MPSIII, examine the long-term clinical consequences, including whether or not the stem cells effectively reduce the stored heparan sulfate.

This strategy depends not only on getting a sufficient number of cells to survive and grow after transplantation but also on the levels of corrective enzymes these cells are able to produce. Dr. Yu and his collaborators are currently determining whether these transplanted cells are capable of making sufficient amounts of the corrective enzyme. In addition, they have genetically engineered these cells to boost their capability of producing the corrective enzyme, improving their therapeutic value.

In preparing these stem cells for use in humans, Dr. Yu has established the first state-supported placenta-umbilical cord blood bank in Georgia. Umbilical cord blood is rich in stem cells that can be harvested for clinical use. This bank is supported by funds provided by other sources, including the Medical College of Georgia and the State of Georgia. Dr. Yu expects that in the future there will be sufficient numbers of stem cells for the treatment of a variety of diseases, such as Sanfilippo disease.

Svitlana Garbuzova-Davis, Ph.D., D.Sc.

Center for Aging & Brain Repair, Department of Neurosurgery, University of South Florida

During the last few years, our research group at the University of South Florida has studied the potential of human umbilical cord blood (hUCB) stem cells to treat various neurodegenerative disorders, as well as brain and spinal cord injuries. Data suggest that these cells are able to repair a damaged or defective nervous system.

In Sanfilippo Syndrome type B, a deficiency of the *Naglu* enzyme leads to accumulation of heparan sulfate. Our results demonstrated that a single intravenous administration of hUCB cells to Sanfilippo mice at different stages of disease (early symptomatic or late stage) had a beneficial effect, probably due to enzyme delivery into these enzyme-deficient mutant mice. These previously shown behavioral improvements, indicating advantages of administered hUCB cells, were supported by our results on the survival, distribution, and differentiation of the transplanted cells. After intravenous administration of hUCB cells, the cells were found widely distributed within and outside the central nervous system. Although many cells were associated with blood vessels, confirming that transplanted cells were still present in the blood circulation, some cells were found in the brain and peripheral organs. These latter cells within the brain tissue may also express neural proteins, and therefore become neural. Interestingly, our results also showed that heparan sulfate accumulation was significantly reduced in the liver and spleen of *Naglu* mice, mainly in females, 6 months after intravenously receiving hUCB cells. Additionally, an anti-inflammatory effect by hUCB cell transplantation was determined.

However, most observed behavioral benefits in Sanfilippo mice were limited to the first months after transplantation, possibly due to a declining production of the missing enzyme over time. To address this

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RESEARCH UPDATE

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limitation, it was necessary for us to investigate the effect of repeated hUCB cell infusions during the disease over time.

The aim of our pre-clinical translational study was to determine the effect of multiple intravenous transfusions of hUCB cells into a mouse model of Sanfilippo type B. We investigated the ability of repeated hUCB cell administration to ameliorate/prevent behavioral dysfunction in mutant mice.

As we showed previously, the Sanfilippo mice demonstrated early hypoactivity (3 months of age, early symptomatic stage of disease) with impaired learning (mostly in males) followed by hyperactivity and memory impairment with aging (8-9 months of age) of both sexes. Multiple administrations of hUCB cells into mutant mice showed improved behavioral activity of both sexes with increased repeat cell transplants. Interestingly, learning improved in mutant *males* treated multiple times mainly at the 3rd and 4th months after cell grafting; whereas their ability to remember (memory) tended to improve at 5th and 6th post-transplant months. During learning, Sanfilippo *female* mice treated multiple times showed learning improvement at the 5th and 6th months after transplantation, although their improved memory was observed early, in the 3rd month post-transplant. The behavioral results were supported by analysis for the presence of transfused hUCB cells in the blood circulation of mutant mice, showing increasing cell numbers after each cell injection.

The data collected so far are very encouraging; however, our current project addresses a number of areas needing further study. It is necessary to determine if hUCB cells reduce the accumulation of heparan sulfate in mutant mice after intravenous multiple vs. single administrations of hUCB cells. Additionally, we will investigate survival, distribution, and specific immunophenotypes of the transfused cells. Also, analysis of the condition of the brain will be performed, since this is an important criterion for evaluating/confirming the nature of the significant beneficial effects of hUCB cells in Sanfilippo mice.

The repeated administration of hUCB cells into the blood circulation of Sanfilippo mice may lead to the development of a new strategy for enzyme replacement for Sanfilippo. Our therapeutic tactic of continuous delivery of the missing Naglu enzyme by multiple cell administrations may be critical to developing a cell transplant strategy.

We Need A Boost

Donations to the Foundation have been below levels of past years. Please consider donating to ensure the Foundation is able to continue its "boost for a cure" and fund all the research projects that show such great promise.

Give Kirby a boost! Your generosity will make all the difference.

Please send donations now to:

The Children's Medical Research Foundation, Inc.
P. O. Box 70
Western Springs, IL 60558

Kirby and all the children afflicted with Sanfilippo thank you.

Kirby gets a boost from her sister, Maggie.

FUNDRAISING OPPORTUNITIES

A Match For Kirby

Does your company have a matching gift program? It could double your support of the Foundation.

Our thanks to **Vito Ugenti and the HSBC Matching Gift Program**.

United Way Can Be For Kirby, Too

Does your company have United Way pledges at your workplace? Although we are not a United Way member, you can designate The Children's Medical Research Foundation as your recipient, and the funds will be forwarded to us through the United Way Campaign! Our United Way Chicago "Agency Number" is 3025558. For those of you out of state wishing to participate, please supply your local United Way agency with the Foundation name, address and our Federal ID #36-4033667.

Our thanks to **the employees of UPS of Los Angeles, CA, Adams & Knight of Hartford, CT, SBC of Chicago and Deerfield High School.**

Celebrate, And Make It For Kirby

Is there a special birthday coming up for a family member or friend? Are you looking for an alternative to the typical "over the hill" gift? Be different. In lieu of gifts, donate to The Children's Medical Research Foundation. Kirby always loves a party!

A Gift Like No Other

Next holiday season give clients a donation to The Children's Medical Research Foundation in their name. It's a gift that won't gather dust and goes far beyond any other.

Go Kasual For Kirby

Tired of wearing those heels, hose or ties five days a week? Why not suggest a **"Go Kasual for Kirby Day"** to your employer? It's a great way to give a "relaxing feel" to a workday and raise funds for the Foundation. Simply send a memo to co-workers explaining the day and set the "fee" to participate.

Still in school but want to help? How about a **"Hat Day"** done the same way! It's fun for the kids and a great way for them to participate in a good cause. No "fee," just leave an amount up to them.

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