

# SBP Scientific Sessions and Poster Session

## Abstracts and Biographies

*In order of appearance in the Program*

### KEYNOTE ADDRESS

## 21st Century Medicine Will Transform Healthcare

**LEROY HOOD, M.D., PH.D.**

President & Co-Founder, Institute for Systems Biology;  
Senior Vice President & Chief Science Officer, Providence  
Health & Services

**Biography:** Dr. Leroy E. Hood graduated from the Johns Hopkins University School of Medicine in 1964 with an M.D. and from Caltech with a Ph.D. in biochemistry in 1968. After three years as a Senior Investigator at NIH, his academic career began at Caltech, where he and his colleagues developed the DNA gene sequencer and synthesizer, and the protein synthesizer and sequencer—four instruments that paved the way for the successful mapping and understanding of the human genome.



A pillar in the biotechnology field, Dr. Hood has played a role in founding fifteen biotechnology companies including Amgen, Applied Biosystems, Integrated Diagnostics and Arivale. He is a member of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. Of the more than 6,000 scientists world-wide who belong to one or more of these academies, Dr. Hood is one of only fifteen people nominated to all three.

Dr. Hood has co-authored numerous textbooks in biochemistry, immunology, molecular biology and genetics, as well as a popular book on the human genome project, *The Code of Codes* and he is just finishing up a text on systems biology. He is the recipient of numerous national and international awards, including the Lasker Award for Studies of Immune Diversity (1987), the Kyoto Prize in advanced technology (2002), the Heinz Award for pioneering work in Systems Biology (2006), and the coveted NAE 2011 Fritz J. and Delores H. Russ Prize for developing automated DNA sequencing.

In addition to having received 17 honorary degrees from prestigious universities in the U.S. and abroad, Dr. Hood has published over 750 peer-reviewed articles and currently holds 36 patents. In 2013, he received the National Medal of Science from President Obama. Hood has been named by *The Best Schools* as one of the 50 Most Influential Scientists in the World Today (2014) <http://isb.io/top50>. *Scientific American* has named Hood as one of the top 6 in their selection of 100 biotech visionaries world-wide (2015) <http://isb.io/visionary>.



## Session 1

### **PMM2-CDG: OVERVIEW, MODELS AND THERAPIES**

Chair: Marc Patterson, M.D., Professor of Neurology, Pediatrics and Medical Genetics, and Chair of the Division of Child and Adolescent Neurology, Mayo Clinic

**FRIDAY, FEBRUARY 23, 2018 9:00 A.M.-12:15 P.M.**



## Natural History of PMM2-CDG



**LYNNE WOLFE, M.S., CNRP, BC**

Senior Nurse Practitioner, NIH/NHGRI, NIH Site Coordinator, Undiagnosed Diseases Program, Study Coordinator, Epi-743 MitoWorks Study and Congenital Disorders of Glycosylation Protocol

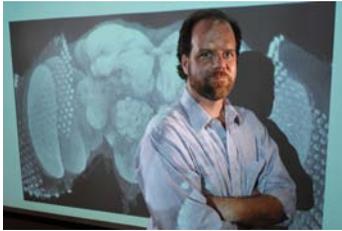
**Abstract:** n/a

**Biography:** Lynne has been a Nurse for over 30 years and a Nurse Practitioner working with children and adults who have all types of Inborn Errors of Metabolism and Mitochondrial diseases for 25 years.

Lynne is a Senior Nurse Practitioner, Associate-Investigator, and the Undiagnosed Diseases Network NIH-UDP Site Coordinator. She is also an Associate-Investigator and Study Coordinator for the Epi-743 Investigational Drug trial and the Congenital Disorders of Glycosylation Natural History study at National Human Genome Research Institute. Her areas of research include: Congenital Disorders of Glycosylation, Mitochondrial disease/dysfunction, treatment of rare diseases, nutrition and supplement support for metabolic and mitochondrial diseases, and transitional care, all areas she has also published in. She speaks frequently to professional and family support groups around the world.



## Coordinated movement, neuromuscular synaptogenesis and trans-synaptic signaling defects in a new *Drosophila* PMM2-CDG disease model



**KENDAL S. BROADIE, PH.D.**

Stevenson Professor of Neurobiology, Professor of Biological Science, Cell & Developmental Biology and Pharmacology, Vanderbilt University and Medical Center

**Abstract:** For the past 5 years, we have been exploring glycan mechanisms modulating neuromuscular synapse formation and plasticity, driven in large part by a *Drosophila* transgenic RNAi screen aimed at identifying novel glycan roles in sculpting synaptic architecture and neurotransmission strength. Several CDG associated genes were identified in this genetic screen, revealing critical roles in synaptogenesis. In this seminar, I focus particularly on a new *Drosophila* PMM2-CDG disease model arising from this work, which displays strongly disrupted glycosylation in the nervous system with global coordinated movement impairments leading to early lethality. Analyses of the glutamatergic neuromuscular junction (NMJ) show severe synaptic glycosylation defects causing impaired structural and functional synaptic development, revealed by confocal and electron microscopy imaging and two-electrode voltage-clamp electrophysiology recording. Mechanistically, PMM2 loss disrupts Wnt trans-synaptic signaling via loss of a Wnt heparan sulfate proteoglycan (HSPG) co-receptor and an extracellular matrix metalloproteinase (MMP). MMPs interact with the HSPG glypican Dally-like protein (Dlp) to regulate Wnt *trans*-synaptic signaling that drives neuromuscular synaptogenesis in an activity-dependent mechanism. Neuronal activity acutely upregulates MMP/Dlp co-localization at the synaptic interface, with Dlp bidirectionally controlling synaptic MMP levels. Dlp glycosaminoglycan (GAG) chains mediate activity-dependent MMP regulation in a mechanism dependent on PMM2 function. This work defines a mechanistic axis from neuronal activity to HSPG-dependent MMP regulation of Wnt *trans*-synaptic signaling that drives activity-dependent neuromuscular synaptogenesis. This new *Drosophila* PMM2-CDG disease model provides a novel means to dissect synaptic impairments causing loss of coordinated movement, and a new avenue to discover and test novel therapeutic treatment strategies directed against the disease state.

**Biography:** I am a molecular geneticist and developmental cell biologist with an intensive research focus on synapse biology, including the mechanisms of 1) synapse development (synaptogenesis), 2) function (neurotransmission) and 3) use/activity-dependent modulation (plasticity). I trained first at the Institute of Neuroscience (ION) at the University of Oregon (Eugene), and then at the University of Cambridge in England. I have since run my own research lab for 25 years at the University of Cambridge (England), University of Utah (Salt Lake City) and Vanderbilt University (Nashville). I am currently the Stevenson Professor of Neurobiology at Vanderbilt, and a professor in the departments of biological sciences, cell & developmental biology, and pharmacology. My research lab marries many traditionally distinct fields and approaches in the study of synapse biology; including genetics (classical screens, molecular genetics, genomics, optogenetics), electrophysiology (whole-cell patch-clamp, two-electrode voltage-clamp), confocal live imaging (protein/membrane movement, Ca<sup>2+</sup> signaling, voltage dynamics), electron microscopy (synaptic ultrastructure) and a range of behavioral assays. In addition to study of normal synapse development, function and plasticity, my lab has developed genetic models for a range of neurological disease states, including neurodegenerative disorders, epilepsies, metabolic disorders, intellectual disabilities, autism spectrum disorders, muscular dystrophies and movement disorders. I became engaged in congenital disorders of glycosylation (CDG) research through the identification of glycan mechanisms in neuromuscular synaptic development, which suggested a mechanistic link to CDG movement impairments. In particular, a primary focus has been *trans*-synaptic signaling in normal and disease state synaptogenesis, and its regulation by glycan mechanisms in the extracellular synaptomatrix.



## Lineage-dependent variations in glycosylation: insights from CDG iPSC models



**RICHARD STEET, PH.D.**

Professor of Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, University of Georgia, Treasurer, Society for Glycobiology

**Abstract:** n/a

**Biography:** A native of upstate New York, Dr. Steet earned his B.A from Colgate University in 1994 and received his Ph.D. from the University of Colorado-Boulder in 2000. During his postdoctoral studies in the laboratory of Dr. Stuart Kornfeld at Washington University School of Medicine in St. Louis, Dr. Steet was involved in the first characterization of a CDG caused by defects in the Golgi-localized COG complex and helped to establish methods to rapidly identify other COG cases. He began his independent research career at the Complex Carbohydrate Research Center on the University of Georgia campus in 2006 and was promoted to Associate Professor in 2012 and Full Professor in 2017. Dr. Steet's laboratory has pioneered the use of zebrafish to study the developmental pathophysiology of glycosylation-related disorders, with a primary focus on mucopolipidosis II and PMM2-CDG. His research interests have also expanded into the use of iPS and iPS-derived cells, as well as chemical glycobiology approaches, as a means to identify specific glycoproteins and pathways that are sensitive in the context of CDGs. Dr. Steet is moving his laboratory to the Greenwood Genetic Center in Greenwood, SC in August 2018 where he will serve as the Director of Research and continue to focus his efforts investigating the pathogenesis and treatment of rare diseases. Dr. Steet is active in the areas of lysosomal storage disorders (where he serves on the Professional Advisory Board for the Mucopolysaccharidoses (MPS) Society and the International Society for Mannosidosis and Related Disorders) as well as glycobiology (where he serves as Treasurer of the Society for Glycobiology).



## Lipo-M1P as a potential therapy for PMM2-CDG



**PATRICE RIOUX, M.D., PH.D.**  
Chief Medical Officer, Glycomine Inc.

**Abstract:** n/a

**Biography:** Dr. Patrice Rioux has been deeply involved in development of drugs for rare diseases for the last 20 years. His background includes development of drugs and biologic products for various indications across neurodegenerative diseases, immunology, pain management, oncology, and metabolic diseases. He was most recently the Senior Vice President of Global Clinical Development at ArmaGen, Inc., a company focused on the development of fusion proteins for the treatment of lysosomal storage diseases, and before that, he was the Chief Medical Officer for Raptor Pharmaceuticals, Inc., where he was responsible for securing regulatory approval of a delayed-release cysteamine for the treatment of a lysosomal storage disease, nephropathic cystinosis, in both the U.S. and Europe. He previously served as Chief Medical Officer at Edison Pharmaceuticals, and as Vice President Clinical at Repligen, where he gained significant orphan disease experience in mitochondrial diseases as well as in autism and auto-immune diseases. After several years as a clinical researcher at INSERM (France), he started his career in the pharmaceutical industry at Biogen, working on multiple sclerosis, before joining Variagenics, Inc., one of the first pharmacogenomic companies. Dr. Rioux received his Medical Education at Faculté de Médecine Pitié-Salpêtrière, his Ph.D. in Mathematical Statistics at Faculté des Sciences, and his Degree of Pharmacology (pharmacokinetics and clinical pharmacology) at Faculté de Médecine Pitié-Salpêtrière.



## Perspectives on ALG9-CDG



**DUNCAN WEBSTER, M.D.**

CDG Parent and Advocate; Associate Professor, Faculty of Medicine, Dalhousie University; Internal Medicine & Infectious Diseases Consultant and Medical Microbiologist, Saint John Regional Hospital, Saint John, New Brunswick

**Abstract:** As a parent, physician and researcher, CDG can be viewed from many perspectives. As a parent and physician, first and foremost, I struggle with the day-to-day challenges of raising a child who has severe deficits due to ALG9-CDG. This presentation will touch on personal experiences relating to my daughter Maria's diagnosis and the challenges that our family has faced. As parents, we often feel helpless and look for positive ways to combat a difficult situation. In this setting, our family established Foundation Glycosylation (the FoG). The FoG supports research for the development of therapies targeting CDG, helps raise awareness of the disorder, and advocates for individuals living with these rare enzyme deficiencies. As a physician and researcher, through the FoG I have been able to recruit and support colleagues in Atlantic Canada to join the fight and raise the profile of glycobiology. In this presentation, I will also share results from our work. In one study we examined intracellular signaling pathways related to autophagy and lysosomal function in dermal fibroblasts from a healthy control and two patients with ALG9-CDG using transcriptomic and proteomic analysis. Compared to healthy fibroblasts, protein synthesis executor mTOR, was upregulated in ALG9-CDG fibroblasts at baseline and down-regulated in the starved state. TFEB, the master regulator of lysosomal biogenesis and many autophagy proteins, was down-regulated in ALG9-deficient fibroblasts, both at baseline and under conditions of stress. With down regulation of TFEB, lysosome biogenesis is disrupted, thereby disrupting autophagy. Cathepsin B belongs to a family of lysosomal cysteine proteases and plays an important role in intracellular proteolysis. Cathepsin B activity was lower at baseline in the ALG9-deficient cells. Furthermore, activity did not increase appropriately in the starved state, signifying faulty lysosomal function and dysfunctional autophagy. ULK1, a regulator of macroautophagy activated under conditions of nutrient deprivation by several upstream signals, was upregulated in the ALG9-deficient fibroblasts, corresponding with a stressed cell even at baseline. Our data characterized lysosomal dysfunction and abnormalities of autophagy at the molecular level. These findings help to explain some clinical complications associated with the ALG9-CDG and underscore the importance of N-linked glycosylation in health and disease.

**Biography:** Duncan graduated from Mount Allison University in 1993 with a BSc in chemistry, biology & physics as well as a BA (honours) in philosophy and religion. He completed his master's degree in philosophy at the University of New Brunswick in 1998 and graduated from Dalhousie Medical School in 2001. He completed his Internal Medicine residency training and Infectious Diseases fellowship at the University of Alberta before returning to Dalhousie University for Medical Microbiology fellowship training which he completed in 2007. He subsequently returned to his home in Saint John, New Brunswick where he continues to work as an infectious diseases consultant at the Saint John Regional Hospital through the Department of Medicine with a cross-appointment in the Department of Laboratory Medicine. Duncan has been named an honorary research associate with the University of New Brunswick. He is an associate professor with Dalhousie University and an active teacher with Dalhousie Medical School providing introductory lectures and clinical skills teaching at Dalhousie Medicine New Brunswick and has served as a preceptor for numerous medical students, clinical clerks and residents in the clinical setting as well as in research activities. He has been the recipient of numerous professional and community awards and citations including the Dalhousie Medical Alumni Association 2012 Young Alumnus of the Year. He has numerous peer-reviewed publications and active research interests in the areas of glycosylation, harm reduction, tuberculosis and zoonoses.



## **Session 2**

# **OTHER GLYCOSYLATION DISORDERS: NOVEL APPROACHES**

**Chair:** Hudson Freeze, Ph.D., Professor of Glycobiology and Director, Human Genetics Program, Sanford Children's Health Research Center, SBP

**FRIDAY, FEBRUARY 23, 2018 1:15 P.M.-4:00 P.M.**



## Novel approaches to identify and validate rare disease genes



**JOSEF PENNINGER, M.D., PH.D.**

Scientific Director, IMBA, Institute for Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria

**Abstract:** n/a

**Biography:** Josef Penninger, M.D., Ph.D., was formerly a lead researcher at the Amgen Research Institute in Toronto. In 2002 he accepted the appointment as founding director of the newly established Institute of Molecular Biotechnology (IMBA) of the Austrian Academy of Sciences in Vienna, Austria. Major achievements include pioneering insights into the molecular basis of osteoporosis and breast cancer, as well as the study of metastatic spread. His group has also developed the first haploid embryonic stem cells for functional genetics. He has authored and co-authored more than 600 scientific papers. Josef Penninger's major awards include the Descartes Prize, the Wittgenstein Prize of the Austrian Federal Government, the Ernst Jung Prize for medical excellence, an AAAS Award the Innovator Award from Era of Hope/DOD and a second ERC Advanced grant.



## Personalized medicine in CDG: An overview of potential therapies



**EVA MORAVA, M.D., PH.D.**

Professor of Pediatrics, Clinical Biochemical Geneticist, Senior Associate Consultant, Department of Clinical Genomics, Mayo Clinic, Editor in Chief Journal of Inherited Metabolic Disease

**Abstract:** MPI-CDG was the first CDG with an effective treatment. Initial case studies showed significant clinical improvement on oral mannose sugar therapy, with less bleeding, diarrhea and blood sugar levels. Mannose, however was not proven to be effective in most patients with PMM2-CDG. Since these therapeutic studies, based on compassionate use, only a few experimental case trials have been initiated, in only a few types of CDG. Oral galactose supplementation improved seizures and a few blood parameters in a subset of SLC35A2-CDG patients, and the bleeding tendency, endocrine function in TMEM165-CDG. Both galactose and manganese improved the seizure disorder in SLC39A8-CDG. Oral fucose treatment improved the immune disorder and decreased infection frequency in a few patients with SLC35C1-CDG. In an observational clinical trial in PGM1-CDG galactose therapy improved the liver function, in some children the growth and some of the hormones and also coagulation abnormalities. N-Acetylmannosamine in trial is shown to be effective in GNE-CDG. In a few patients oral uridine therapy was tried but the effect hereof is not clear. Nucleotide sugars and uridine therapy was not successful in a trial in PGM3-CDG. In CAD-CDG both the severe seizures and the anemia are treatable by oral uridine supplements, based on case studies. Liver transplantation has been performed in MPI-CDG and CCDC115-CDG. Heart transplantation was successful in mild DOLK-CDG. Bone marrow transplantation led to improvement of the immune disease in PGM3-CDG. Future clinical trials aim at D-galactose use in different CDGs, liposomal mannose-1-phosphate and potential chaperone therapy in PMM2-CDG.

**Biography:** Dr. Morava graduated as a Medical Doctor by the University of Pecs, Hungary. She specialized in pediatrics in 1994. She specialized in human genetics in 1999. Dr. Morava defended her Ph.D. thesis on Molecular cytogenetic investigations in intellectual disability syndromes in 2000. She trained additionally in clinical biochemical genetics at Tulane University between 1996-1998, and worked as a clinical geneticist until 2002, and as a metabolic pediatrician at umcRadboud in the Netherlands till 2012. Since 2012 she has been full professor at the Tulane University Medical Center, at the Hayward Genetics Center, as a biochemical geneticist. Since 2015 she is also faculty at the University Hospitals Leuven, in Belgium. Eva Morava is a member of national and international committees and scientific advice groups, including the SSIEM council. Her list of publications includes more than 240 peer reviewed scientific papers.

Her special research interests are translational metabolism and congenital disorders of glycosylation (CDG). Her current focus is on PGM1 deficiency and on developing new therapies in CDG. She has a strong collaboration with European universities. She is editor in chief of the Journal of Inherited Metabolic Disorders. Dr Morava is a collaborator of the international network CDG & Allies – PPAIn. Dr Morava shares her expertise and knowledge as a member of the Advisory Committee (CDG-CARE), as a board member of the Thallingh Roorda Foundation and as the vice coordinator for MetabERN & coordinator for the sub-network glycosylation disorders.



## Peters Plus Syndrome: A congenital disorder of glycosylation



**ROBERT S. HALTIWANGER, PHD**

GRA Eminent Scholar in Biomedical Glycosciences  
Editor-in-Chief, Glycobiology  
Professor of Biochemistry and Molecular Biology  
Complex Carbohydrate Research Center  
University of Georgia

**Abstract:** Peters plus syndrome (PPS) is a rare genetic disorder characterized by anterior eye segment abnormalities, short stature, brachydactyly, and developmental delay. In addition, cleft palate, congenital heart defects, and/or urogenital defects are present in 50% of patients. The disease is caused by recessive loss-of-function mutations in  $\beta$ 3-glucosyltransferase (B3GLCT). B3GLCT transfers a glucose to O-fucosylated thrombospondin type I repeats (TSRs). TSRs with O-fucosylation consensus sequences are tandemly repeated within 49 predominantly extracellular matrix (ECM) associated proteins. The ADAMTS class of proteins (A Disintegrin and Metalloproteinase with ThromboSpondin motifs) makes up nearly 50% of these proteins, and is implicated in controlling the structural properties of the ECM, influencing cell migration, organogenesis, tissue organization and cell signaling. We are using a mouse *B3glct* knockout to gain insight into the developmental origin of PPS and identify B3GLCT targets responsible for PPS anomalies. *B3glct* mutants showed reduced neonatal viability. MicroCT and MRI imaging identified potential ventricular septal and myocardial wall defects in some homozygotes. The survivors were runted and had broadened and domed heads. Skeletal preparations, 3D microCT renderings, and histological analyses identified defects in cranium structure, endochondral ossification, and hydrocephalus. Finally, reducing the copies of *Adamts9* in *B3glct* homozygotes resulted in 100% neonatal lethality. These results provide evidence that defects in PPS patients result, at least in part, from abnormalities in ADAMTS9 function, and demonstrate that the *B3glct* mutant mouse will provide an invaluable resource for understanding how changes in the ECM structure or composition can lead to the collection of common congenital abnormalities seen in PPS patients.

**Biography:** Robert Haltiwanger did his doctoral work with Dr. Robert L. Hill at Duke University Medical School and his postdoctoral work with Dr. Gerald Hart at Johns Hopkins University School of Medicine. He joined the Department of Biochemistry and Cell Biology at Stony Brook University as an Assistant Professor in 1992 and rose through the ranks to become Chair in 2007. In 2015 he moved to the Complex Carbohydrate Research Center at the University of Georgia where he is currently the Georgia Research Alliance Eminent Scholar in Biomedical Glycosciences. He is a past-President of the Society for Glycobiology and is currently Editor-in-Chief of the Society's journal, Glycobiology. He and his colleagues work on unusual O-linked carbohydrate modifications found on small cysteine-rich protein modules: epidermal growth factor-like (EGF) repeats and thrombospondin type 1 repeats (TSR). They were the first to report the existence of O-fucose and O-glucose glycans on the EGF repeats of the Notch receptor and have played key roles in identifying the enzymes responsible for the addition of these glycans including Fringe, Protein O-fucosyltransferase 1 (POFUT1), and Rumi. These glycans play essential roles in Notch function and are known modulators of Notch activity. TSRs are fucosylated by a distinct enzyme, POFUT2, and the O-fucose is extended by  $\beta$ 3-glycosyltransferase (B3GLCT). Mutations in B3GLCT cause Peters Plus Syndrome, a rare genetic disorder characterized by anterior eye chamber defects, short stature, brachydactyly, and developmental delay. The Haltiwanger laboratory examines how these glycans affect the function of the proteins they modify.



## Mechanistic insights and therapeutic approaches for O-glycosylation-deficient muscular dystrophy



### KEVIN P. CAMPBELL, PH.D.

Investigator, Howard Hughes Medical Institute, Roy J. Carver Biomedical Research Chair in Molecular Physiology and Biophysics, Executive Officer of the Dept. of Molecular Physiology and Biophysics Director, Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center at the Carver College of Medicine, University of Iowa

**Abstract:** O-glycosylation-deficient muscular dystrophy are neuromuscular diseases in which the aberrant post-translational modification of dystroglycan results in loss of an essential link between  $\alpha$ -dystroglycan and its laminin-G domain-containing extracellular matrix (ECM) ligands. Recent genetic data has shown that mutations in at least eighteen genes encoding post-translational enzymes lead to a reduced Xyl-GlcA disaccharide repeat on dystroglycan and cause congenital/limb-girdle muscular dystrophies, which can be accompanied by brain and eye abnormalities. Our previous efforts to understand the molecular mechanism underlying the ability of  $\alpha$ -DG to bind the ECM revealed that LARGE is a bifunctional enzyme with both xylosyltransferase (Xyl-T) and glucuronyltransferase (GlcA-T) activities, and that it generates a novel heteropolysaccharide [-GlcA- $\beta$ 1,3-Xyl- $\alpha$ 1,3-]<sub>n</sub>. However, how the LG domains of laminin and other proteins recognize specifically the LARGE product remained a mystery. Using a novel enzymatic method of digestion, we demonstrated that native  $\alpha$ -DG from skeletal muscle contains the unmodified heteropolysaccharide [-GlcA- $\beta$ 1, 3-Xyl- $\alpha$ 1,3-]<sub>n</sub> and that its presence confers the ability to bind laminin. We have also used a multidisciplinary approach involving NMR binding studies and crystallographic analysis of the laminin LG4-5 region bound to a LARGE-synthesized oligosaccharide to determine the structural basis of high-affinity binding of laminin to  $\alpha$ -DG. Our results reveal a novel mechanism of carbohydrate recognition. Moreover, they provide a structural framework for elucidating the mechanisms that underlie the dystroglycanopathies as well as providing insights for the development of therapeutic approaches for treating these diseases.

**Biography:** Dr. Kevin P. Campbell is an investigator with the Howard Hughes Medical Institute, the Roy J. Carver Biomedical Research Chair in Molecular Physiology and Biophysics, Executive Officer of the Department of Molecular Physiology and Biophysics, and Director of the Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center at the Carver College of Medicine, University of Iowa. Dr. Campbell is internationally recognized for his discovery of dystroglycan, and for elucidation of its function as an extracellular matrix receptor. He established that dystroglycan is involved in a variety of physiological and developmental processes, including maintenance of skeletal muscle function as well as formation and function of the central nervous system. He showed that complex post-translational processing of dystroglycan, including extensive glycosylation, is required for its ability to function as an extracellular matrix receptor that abnormal glycosylation results in a variety of congenital and limb-girdle muscular dystrophies with or without associated defects in brain development and function. Dr. Campbell is an elected member of the Institute of Medicine, the National Academy of Sciences, and the American Academy of Arts and Sciences. Finally, Dr. Campbell has been chosen as the 2017 Society for Glycobiology President's Innovator Award winner.



## Genetic knockout screens reveal a critical role for glycosylation in infectious disease



**JAN CARETTE, PH.D.**

Assistant Professor, Department of Microbiology and Immunology,  
Stanford University

**Abstract:** Viruses and their human host are in a constant battle where the viruses hijack cellular components to facilitate their replication and spread, while the host expresses genes to fight of the infection. We study this co-evolutionary battle between host defense proteins and viral proteins using advanced genetic tools. In this approach, we systematically inactivate each human gene individually and measure how this affects viral infection. In this way, we can identify the proteins that are most critical for virus infection. Using this approach we have discovered that Ebola virus infection absolutely requires NPC1, a protein that normally is involved in cholesterol transport. We found an important role for glycosylation in the entry process of several viruses that utilize different glycans to attach to cells and gain entry. Examples include alpha-dystroglycan for the hemorrhagic fever Lassa virus and sialic acid for enterovirus D68 infection. In a comparative genetic screen for cellular components that are required for the replication of dengue virus and hepatitis C virus, we uncovered a surprising role for the oligosaccharyltransferase (OST) complex. We found that the OST complex plays an essential role in the replication of several mosquito-borne flaviviruses including dengue virus and Zika virus. Inhibition of the OST complex using a small molecule inhibitor led to severely reduced replication levels suggesting that the OST complex is a viable target for antiviral therapy.

**Biography:** Dr. Jan E. Carette is Assistant Professor in the Department of Microbiology and Immunology at Stanford University, California, USA. He received his doctorate from Wageningen University, The Netherlands, and did his postdoctoral training at Vrije Universiteit Amsterdam, The Netherlands, and at the Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, USA. His laboratory uses genetic approaches to understand the molecular mechanisms of virus–host interactions, ranging from pathogenic viruses to viruses used in gene therapy.



## Novel glycosylation disorders



### **HUDSON FREEZE, PH.D.**

Professor of Glycobiology and Director, Human Genetics Program, Sanford Children's Health Research Center, Sanford Burnham Prebys Medical Discovery Institute (SBP)

**Abstract:** n/a

**Biography:** Hudson Freeze, Ph.D., is Professor of Glycobiology and Director of the Human Genetics Program at Sanford Burnham Prebys Medical Discovery Institute (SBP), as well as an Adjunct Professor at the UCSD School of Medicine. Dr. Freeze's early work in the late 1970's centered on understanding lysosomal enzyme targeting related to human I-cell disease, and later (1995-on) refocused on Congenital Disorders of Glycosylation (CDG). He has worked in Glycobiology for over 40 years—the last 22 focused on identification and understanding of human glycosylation disorders. Alone or in collaborations, the Freeze lab has now discovered **21** human glycosylation disorders. At present, it is the only laboratory in the United States primarily devoted to studying CDG.

In recent years, Dr. Freeze and his lab have used phosphomannose isomerase (Mpi) knock-out and hypomorphic mouse models for MPI-CDG (CDG-Ib) to understand the pathology and effect of mannose therapy. Recently, the Freeze lab's study in Mpi-hypomorphic mice showed that mannose supplementation could have adverse effects during pregnancy. The lab used stable isotope and radio-labeling to analyze the metabolic flux of mannose and other sugars. These studies were named "Paper of the Year" for 2014 by The Journal of Biological Chemistry.

Dr. Freeze recently served as President of FASEB (2016-2017), a 125,000-member alliance of biomedical researchers. Dr. Freeze is also a Past President of the Society for Glycobiology (SfG) and its first representative appointed to the FASEB Board of Directors. Dr. Freeze isolated the first extreme thermophile, *Thermus aquaticus* (*Taq*), for which he won the 2013 Golden Goose Award.



## Session 3

### **NGLY1: A DISORDER OF DE-GLYCOSYLATION**

**Chair:** Randal Kaufman, Ph.D., Director and Professor, Degenerative Diseases Program, Neuroscience and Aging Research Center, Sanford Burnham Prebys Medical Discovery Institute (SBP)

**SATURDAY FEBRUARY 24, 2018, 9:00 A.M.-12:15 P.M.**



## Precision medicine and NGLY1



**MATTHEW MIGHT, PH.D.**

Director of the Hugh Kaul Precision Medicine Institute, Hugh Kaul Endowed Chair of Precision Medicine, Professor of Medicine & Professor of Computer Science, University of Alabama at Birmingham

**Abstract:** Precision medicine requires an algorithmic approach to the delivery of care, and it encounters a wide range of computational challenges. This talk will center on an in-depth case study in precision medicine, highlighting computational challenges at the forefront of the field.

**Biography:** Dr. Might is the Inaugural Director of the Hugh Kaul Precision Medicine Institute, the Hugh Kaul Endowed Chair of Personalized Medicine, and both a Professor of Medicine and Professor of Computer Science at the University of Alabama at Birmingham. Dr. Might has served as a Strategist in the Executive Office of the President at the White House for both the prior and current administration. He is the Chief Science Officer of NGLY1.org and a Co-Founder and Scientific Advisor to Pairnomix, LLC. He was previously an Associate Professor of Computer Science and Adjunct Associate Professor of Pharmaceutical Chemistry at the University of Utah. He received his Ph.D. in Computer Science from Georgia Tech in 2007. He tweets from @mattmight and blogs at [blog.might.net](http://blog.might.net).



## Discovering NGLY1 therapeutics: A research collaboration between NCATS, [NGLY1.org](http://NGLY1.org) and Retrophin

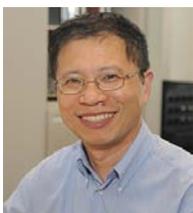
Abstract: n/a



**STEVE RODEMS, PH.D.**

Senior Director, Discovery Biology, Retrophin

**Biographies:** **Steve Rodems, Ph.D.** is Senior Director, Discovery Biology at Retrophin, a biopharmaceutical company headquartered in San Diego that specializes in identifying, developing and delivering life-changing therapies to people living with rare diseases. In his current role, Dr. Rodems is responsible for identifying and leading external collaborations with academics, non-profits, industry and disease foundations for discovery-stage projects. He also contributes to the evaluation of external clinical-stage assets under consideration for in-licensing or partnering. Dr. Rodems is passionate about finding cures for rare, genetic diseases, and has experience designing and implementing innovative therapeutic approaches using precision medicine-driven strategies and a patient-centric philosophy. He realizes the value of a strong partnership with patient foundations and the patient community throughout the discovery and development life cycle. Dr. Rodems received his Ph.D. in Biochemistry from the University of Wisconsin, Madison, and did a postdoctoral fellowship in the Department of Biology at University of California, San Diego.



**WEI ZHENG, PH.D.**

Leader, Biology, Therapeutics for Rare and Neglected Diseases, Division of Pre-Clinical Innovation, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH)

**Wei Zheng, Ph.D.** received his Ph.D. in pharmacology from the State University of New York at Buffalo, where he studied ion channel pharmacology and completed a postdoctoral fellowship in molecular biology. Prior to joining NIH in 2005, Zheng spent 12 years as a researcher at pharmaceutical companies, including Berlex, Amgen and Merck, where he gained broad experience in drug target and lead discovery in cancer and in cardiovascular, autoimmune, neurodegenerative and infectious diseases. At NIH, Zheng has collaborated on high-throughput screening and probe discovery projects that led to identification of more than two dozen small molecule probes for use as research tools. He also has conducted drug discovery and development research for rare and neglected diseases. Currently, he is leading a group of biologists to support pre-clinical drug development as part of the [Therapeutics for Rare and Neglected Diseases](#) (TRND) program. Zheng also serves as editor-in-chief of the peer-reviewed journal *Current Chemical Genomics and Translational Medicine*.



## Genetic analysis of NGLY1 action in proteasome biology



**GARY B. RUVKUN, PH.D.**

Department of Molecular Biology, Massachusetts General Hospital  
Professor, Harvard Medical School

**Abstract:** n/a

**Biography:** The Ruvkun lab uses *C. elegans* molecular genetics and genomics to study miRNA and RNAi pathways. Using genetic and RNA interference approaches, we have identified many genes that positively or negatively regulate RNAi and microRNA pathways. These genes reveal the trajectory of siRNAs and miRNAs as they target mRNAs, as well as components that may be developed as drug targets to enhance RNAi in mammals.

Over the past decade, we discovered that like mammals, *C. elegans* uses an insulin signaling pathway to control its metabolism and longevity. This analysis has revealed striking congruence of molecular mechanisms at many steps in the pathway, suggesting that insulin regulation of longevity and metabolism is ancient and universal. The new genes of the insulin pathway that have emerged from these studies are conserved in animal phylogeny and represent new targets for diabetes drug development.

Functional genomic analyses using RNAi libraries of every *C. elegans* gene now allows a systematic study of metabolism and aging. Our lab has surveyed 18,000 genes for their action in regulation of longevity, fat deposition, RNAi, miRNA regulation, and molting. This analysis gives a global view of the molecular machines that operate in these pathways. Current research in the Ruvkun lab attempts to weave these lists of aging regulatory genes into pathways that assess and regulate metabolic tempo and mode, repair and regeneration, and protective and degenerative pathways. A neuroendocrinology of energy balance and longevity will emerge from these studies.

We are developing protocols and instruments that use PCR primers corresponding to universal sequence elements of the 16S RNA gene to search for diverse microbes that may cause diseases unsuspected to be due to pathogens and microbes from extreme environments. One long term goal of this project is to send a robotic thermal cycler with these primers to Mars in search of microbial life that is ancestrally related to life on Earth.



## Can basic science contribute to curing human genetic disorders?



### TADASHI SUZUKI, DSCI

Team Leader, Glycometabolome Team, RIKEN Global Research Cluster

Website: [http://www.riken.jp/en/research/labs/grc/sys\\_glycobiol/glycometabolome/](http://www.riken.jp/en/research/labs/grc/sys_glycobiol/glycometabolome/)

**Abstract:** The cytoplasmic peptide:N-glycanase (PNGase) is the enzyme widely conserved throughout eukaryotes. I have been carrying out a basic science research on this enzyme for over a quarter century. Initially many casted a doubt on the functional importance of this enzyme, while now we know that this enzyme is involved in the degradation of misfolded/non-functional glycoproteins destined for the degradation process called ERAD (ER-associated degradation). In 2012, a patient harboring mutations of PNGase gene (*NGLY1*) was first reported. Symptom of these patients includes developmental delay, multifocus epilepsy, involuntary movement and liver dysfunction. From this report, it is clearly suggested that the cytoplasmic PNGase play a pivotal role in normal human development.

We analyzed *Ngly1*-deficient mice and found that they are embryonic lethal in C57BL/6 (B6) background. Surprisingly, the additional deletion of *Engase*, encoding another cytosolic deglycosylating enzyme called ENGase (endo- $\beta$ -*N*-acetylglucosaminidase), resulted in the partial rescue of the lethality of the *Ngly1*-deficient mice. Additionally, we also found that a change in the genetic background of B6 mice, produced by crossing the mice with an outbred mice strain (ICR) could rescue the embryonic lethality of *Ngly1*-deficient mice. Viable *Ngly1*-deficient mice in a B6 and ICR mixed background, however, showed a very severe phenotype reminiscent of the symptoms of *NGLY1*-deficiency subjects. Again, many of those defects were strongly suppressed by the additional deletion of *Engase* in the B6 and ICR mixed background.

In this presentation, I will present our most recent observation on the functional analysis of *Ngly1*-KO mice. I will also some examples how basic science can, unexpectedly, provide the potential therapeutic options for human genetic disorders.

**Biography:** Tadashi Suzuki received D. Sc. (1997) from the Department of Biochemistry and Biophysics, Graduate School of Science, University of Tokyo, Japan. He then became a postdoctoral fellow/research assistant professor at Department of Biochemistry and Cell Biology, State University of New York at Stony Brook (1997-2001). In December 2001 he returned to Japan to start an independent career as a Researcher, Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST) (2001-2005). In February 2002 he became an Assistant Professor at University of Tokyo, Graduate School of Science, and in January 2004 he moved to Osaka to serve as a Visiting Associate Professor, Osaka University, Graduate School of Medicine. Since October 2007, he holds a current position as a Team Leader, Glycometabolome Team, Systems Glycobiology Research Group, RIKEN. He received Genzyme Award for the best Ph. D. thesis in Glycobiology from Society for Glycobiology (1997), Young Investigator Awards from the Japanese Biochemical Society (2005)/the Japanese Society for Carbohydrate Research (2008), and Glycobiology Significant Achievement Award from Society for Glycobiology (2016). He is currently serving as an executive editor on *Biochimica et Biophysica Acta* - General Subjects and an editorial board member of *Glycobiology*.



## Genetic and environmental modulation of NGLY1 deficiency



**CLEMENT CHOW, PH.D.**

Assistant Professor, Department of Human Genetics,  
University of Utah

**Abstract:** Autosomal recessive loss-of-function mutations in *N-Glycanase 1 (NGLY1)* cause *NGLY1* deficiency, the only known human disease of deglycosylation. *NGLY1* deficiency is a devastating, extremely rare, neglected disease. Patients with *NGLY1* deficiency present with developmental delay, movement disorder, seizures, hypotonia, liver dysfunction, and alacrima. *NGLY1* is a conserved component of the endoplasmic reticulum associated degradation (ERAD) pathway. ERAD is responsible for degrading misfolded proteins that accumulate in the lumen of the ER. *NGLY1* deglycosylates misfolded proteins in the cytoplasm as they are translocated from the ER lumen for degradation. While little is known about the pathogenesis underlying *NGLY1* deficiency, it is thought that loss of *NGLY1* activity results in accumulation of highly N-glycosylated misfolded proteins in the cytoplasm, acting as a 'sink' for free UDP-GlcNAc. In turn, this might deplete the circulating pool of UDP-GlcNAc in the cell, resulting in disease. We hypothesized that restoring the levels of UDP-GlcNAc in the cells might rescue some of the phenotypes associated with *NGLY1* deficiency. We used ubiquitous RNAi knockdown of *Pngl* (*Drosophila* ortholog of *NGLY1*) in *Drosophila* to model complete loss of *NGLY1* activity seen in human patients. Using next generation sequencing technology and genetic modifier screens, we have identified several nutritional, environmental, and genetic manipulations that eliminate most lethality associated with this *Drosophila* model of *NGLY1* deficiency. We show that supplementing the normal *Drosophila* diet with GlcNAc can rescue lethality associated with loss of *NGLY1* activity. We also demonstrate that genetic alterations in ERAD and cytoplasmic heat shock pathways can influence the lethality of *NGLY1* knockdown. These data suggest a plausible pathophysiology for *NGLY1* deficiency. More importantly, our study points to a potential therapy through a simple, readily available, diet supplement.

**Biography:** Clement received his BA from Cornell University in 2003. He completed his Ph.D. in 2008 in the Department of Human Genetics at the University of Michigan, where he identified and studied a new form of Charcot Marie Tooth disease, a rare peripheral neuropathy. Clement completed his postdoctoral training at Cornell University. The Chow Lab seeks to understand the role of genetic variation on disease outcomes. The lab employs quantitative and functional tools, in a variety of model organisms, to study how genetic variation impacts basic cellular traits important to rare diseases. Our work in model organisms will help to design precision medicine approaches and therapies.



## Role of NGLY1 in *Drosophila* development



**HAMED JAFAR-NEJAD, M.D.**

Associate Professor, Department of Molecular and Human Genetics,  
Program in Developmental Biology, Baylor College of Medicine

**Abstract:** Mutations in the human *N*-glycanase 1 (*NGLY1*) cause a rare, multisystem congenital disorder with global developmental delay. However, the mechanisms by which *NGLY1* and its homologs regulate embryonic development are not known. We have recently reported that a *Drosophila* gene called *Pngl* encodes an *N*-glycanase enzyme and exhibits a high degree of functional conservation with human *NGLY1*. Loss of *Pngl* results in developmental midgut defects reminiscent of midgut-specific loss of BMP signaling. *Pngl* mutant larvae also exhibit a severe midgut clearance defect, which cannot be fully explained by impaired BMP signaling. Genetic experiments indicate that *Pngl* plays key roles in the mesoderm during *Drosophila* development. Loss of *Pngl* results in a severe decrease in the level of Dpp homodimers and abolishes BMP autoregulation in the visceral mesoderm mediated by Dpp and Tkv homodimers. Thus, our studies uncover a novel mechanism for the tissue-specific regulation of an evolutionarily conserved signaling pathway by an *N*-glycanase enzyme. I will provide an update on our ongoing studies on the function of *NGLY1* during fly development.

**Biography:** Hamed Jafar-Nejad's group is interested in the roles of glycosylation and deglycosylation in the regulation of animal development and in human disease pathogenesis. He received his M.D. from Tehran University of Medical Sciences and learned basic molecular biology techniques in a research institute in Iran. He spent one year in the Neuroscience Research Institute at the University of Ottawa, where he studied the transcriptional regulation of a serotonin receptor implicated in mood disorders. He then moved to Houston and performed his postdoctoral training in the area of Notch signaling and *Drosophila* neurogenesis with Dr. Hugo Bellen at Howard Hughes Medical Institute, Baylor College of Medicine. In December 2006, he joined the faculty at the University of Texas Health Science Center at Houston, focusing on a glycosyltransferase called Rumi, which they had identified in *Drosophila* as a key regulator of the Notch signaling pathway. In 2012, he was recruited back to Baylor, where his group continues their studies on the role of glycosylation in animal development. In recent years, Jafar-Nejad laboratory has devoted significant effort to understanding the function of human rare disease genes in animal development. In one project, the lab has established a mouse model for Alagille syndrome and has identified Rumi (*Poglut1*) as a dominant genetic suppressor of the Alagille biliary phenotypes in the mouse. In another project, they are using *Drosophila* to understand the developmental roles of a deglycosylation enzyme called *N*-glycanase 1 (*NGLY1*), mutations in which cause a multi-system developmental disorder called *NGLY1* deficiency. It is hoped that these collaborative studies will shed light on the pathophysiology of these two diseases and will help identify new therapeutic approaches for them.



## Poster Session Abstracts

*Alphabetical by last name of presenter*

**FRIDAY, FEBRUARY 23, 5:00-7:00 P.M.**

### **Making Glycoproteomics via Mass Spectrometry More Accessible to the Greater Scientific Community**

Marc D. Driessen, Catherine C. Going, Christina M. Woo, Sharon J. Pitteri, and Carolyn R. Bertozzi

**PRESENTED BY MARC D. DRIESSEN, PH.D.**

Bertozzi Lab, Department of Chemistry, Stanford University

We have recently developed a method that, for the first time, is capable of facilitating both, glycan structure and attachment site analysis for both N- and O-glycans alike. This method named '*Isotope Targeted Glycoproteomics*' (IsoTaG) is based on the introduction of an isotopic label that also functions as a (cleavable) enrichment handle. It allows for enrichment of metabolically labelled glycoproteins. We found an unprecedented enrichment and detection of low abundant glycoproteins without either the need to truncate glycan structures or complex sample fractionation.

We aim to make this method a widespread tool for both, glycoproteomics experts and non- experts and have begun the transfer of the method to interested laboratories by supplying them with a novel approach to generate meaningful glycoproteomic datasets. We also plan an evaluation of the IsoTag workflow *via* interlaboratory comparison of identical samples. This small round-robin will be the basis for standardized procedures for an IsoTag 'kit'. These will then be tested for general applicability by dissemination - along with a set of standards - to a larger group of laboratories (mainly MS core facilities). After evaluation these results will be available in the online repository ([www.IsoStamp.org](http://www.IsoStamp.org)).

We also demonstrate the versatility of the IsoTag method through use in additional systems. We are highly interested in applying IsoTag to either primary cells or cell lines with a CDG background as we are convinced that this method has the potential to give new and important insights into these diseases. To this end, actively seek new collaborations.

### **Exploring the clinical and biochemical spectrum of Type I congenital disorders of glycosylation**

**ANDREW C. EDMONDSON, M.D., PH.D.**

Division of Human Genetics and Metabolism, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

**Background:** Recent studies have shown that biomarkers for monitoring protein glycosylation are important in the diagnosis and treatment of type I congenital disorders of glycosylation (CDG). Plasma N-glycans provide a global view of protein glycosylation in multiple tissues, with plasma N-linked Man2-9 species being primarily derived from extrahepatic tissues and N-tetrasaccharide from liver.

**Methods:** We performed a retrospective chart review of CDG patients with eight type I CDG subtypes, who were cared for at the Children's Hospital of Philadelphia. Longitudinal data from the cohort were standardized with the Nijmegen Pediatric CDG Rating Scale (NPCRS). Plasma N-glycan abnormalities



were measured via a novel isotope dilution method by ESI-QTOF with highly enhanced specificity and sensitivity. Patient NPCRS and plasma N-glycan results were compared.

Results: Diagnostic N-glycan profiles were observed in PMM2-CDG, ALG1-CDG, ALG3-CDG, and ALG9-CDG. An increased plasma N-Man5/Man9 ratio was observed in most type I CDG (3.7-16.3; control < 3.5). STT3B-CDG, GMPPB-CDG, and ALG13-CDG patients with normal transferrin profiles exhibited increased N-Man5/Man9 ratios.

Conclusions: Additional characterization of type I CDG patients is necessary to determine the clinical spectrum and natural history. Our cohort suggests that mild or neurologic type PMM2-CDG is underdiagnosed in our population. Plasma N-Man5/Man9 ratio may be an important extrahepatic diagnostic marker for type I CDG, especially when the transferrin profile is normal. Biomarkers for monitoring protein glycosylation in extrahepatic tissues, such as N-glycans, are particularly important as the efficacies of rare sugar therapies and other treatment modalities are studied in therapeutic trials.

## Exploring the role of Nrf1 in NGLy1 deficiency

Ulla I.M. Gerling-Driessen, Frederick M. Tomlin, Yi-Chang Liu, Carolyn R. Bertozzi

**PRESENTED BY ULLA I.M. GERLING-DRIESSEN, PH.D.**

Bertozzi Lab, Department of Chemistry, Stanford University

Recently a rare inherited congenital disorder, N-Glycanase 1 (NGLy1) deficiency, caused by heterozygous inactivating mutations in the *ngly1* gene has been discovered. Patients suffering from NGLy1 deficiency exhibit a spectrum of symptoms, such as global developmental delay, hypotonia, seizures, peripheral neuropathy, alacrima and liver abnormalities. NGLy1 is thought to function as a key component of the ER-associated degradation (ERAD) machinery by catalyzing the de-N-glycosylation of glycoproteins in the cytosol. Interestingly, NGLy1 deficient cells have been found to lack a sufficient proteasome function.

Our hypothesis is that a single NGLy1 substrate might be dependent on de-N-glycosylation for its function and could therefore be the underlying mechanism mediating disease pathologies. The transcription factor Nuclear Factor Erythroid-2 Related Factor 1 (NFE2L1, also called Nrf1), a member of the "cap'n'collar" (CNC) bZIP family is the main regulator of proteasomal subunit gene expression. It is involved in many vital metabolic pathways, and its activation occurs via the ERAD-pathway and involves retro-translocation from the ER to the cytosol. Under basal conditions, Nrf1 is continually targeted for proteasomal degradation. However, under conditions where proteasome activity is compromised, deglycosylated Nrf1 accumulates and exerts its nuclear functions, including transactivation of proteasomal subunit genes (bounce back).

We here show that the correct processing, subcellular localization and activity of Nrf1 is dependent on functional NGLy1. On this basis, we hypothesize that impaired de-N-glycosylation of Nrf1 in the absence of NGLy1 results in an abrogated bounce back response that in turn contributes to the disease symptoms associated with NGLy1 deficiency.

## Three Children, Three Genes, and Two Hexoses

**PHILIP M JAMES, M.D., M.P.H.**

Phoenix Children's Hospital



The greater than 100 types of Congenital Disorders of Glycosylation (CDG) affect every organ system, tissue and fluid. Their myriad phenotypes are not specific to the encoded protein's function or its pathway location. Clinicians rarely consider CDGs in their differential; therefore many patients undergo lengthy evaluations which can delay treatment. Two patients described in this poster have CDGs which are treatable: MPI-CDG (mannosephosphate isomerase deficiency) with D-mannose; and PGM1-CDG (phosphoglucomutase deficiency) with D-galactose. The ALG1-CDG (beta-1,4-mannosyltransferase deficiency) patient underwent a treatment trial with D-mannose.

The boy with ALG1-CDG presented with FTT, microcephaly, epilepsy, cognitive impairment, and multiple coagulation factor deficiencies. D-mannose treatment at 1gm/kg/day resulted in greater than 50% reduction in the abnormal 1124m/z glycan ( $p < 0.001$ ). No hematologic, septic, coagulation or endocrine complications occurred. He gained developmental milestones, spoke phonemes, and visual acuity improved.

The boy with MPI-CDG presented with catastrophic sepsis, iliac vein thrombosis, transaminitis, hypoglycemia and hypoalbuminemia. Liver biopsy demonstrated numerous myelinosomes surrounding bile canaliculi, perisinusoidal fibrosis and macrosteatosis. He did not have FTT or diarrhea. Treatment with 1gm/kg/day of D-mannose has resulted in normalization of glucose, albumin, AT-III, liver transaminases; and 3-4 fold decrease in mono-oligo/di-oligo & a-oligo/di-oligo ratios.

The boy with PGM1-CDG presented with short stature, hypoglycemia elevated liver transaminases and CK; liver biopsy demonstrated steatohepatitis grade 4/8 stage 3. Treatment with 1.5-1.8gm/kg/day of D-galactose has resulted in normalization of glucose, AFP, CK, IGF-I, near normalization of transaminases; and 3-4 fold decrease in mono-oligo/di-oligo & a-oligo/di-oligo ratios. He is no longer listed for liver transplantation.

## **Inherited GPI deficiency: our recent progress**

**YOSHIKO MURAKAMI, PH.D.**

Yabumoto Department of Intractable Disease Research, Research Institute for Microbial Diseases, Osaka University, Japan

Inherited GPI deficiency (IGD) is recently designated as an Intractable Disease by the Ministry of Health, Labour and Welfare of Japan. GPI (glycosylphosphatidylinositol) is a glycolipid which anchors various proteins to the cell surface. There are more than 150 kinds of proteins post-translationally modified with GPI, such as alkaline phosphatase, on human cells. Complete deficiency causes embryonic death because of loss of all the GPI-anchored proteins (GPI-APs) on the cell surface. 27 genes are identified to be involved in biosynthesis and modification of GPI-APs. Among them, there have been 16 kinds of IGDs reported until now. The numbers of the reported individuals with IGD are 30 in Japan and 200 globally. Main symptoms are developmental delay, intellectual disability and seizures and the affected individuals also show very broad symptoms such as characteristic facial features and various anomalies. IGDs can be screened by the decreased expression of CD16, a GPI-AP, on the blood granulocytes. As pyridoxine (vitamin B6) administration is effective to control seizures of some of the individuals with IGD, it is important to diagnose properly and to begin treatment from early age. Here, I will present about our screening system, some new points of IGDs including overlapping diseases, the phenotypic analysis of the mouse model and the possibilities for the treatment of the IGD patients.



## **Impaired Fucosylation Defines Two Novel Types of Congenital Disorders of Glycosylation**

**BOBBY G. NG**

Freeze Lab, Sanford Burnham Prebys Medical Discovery Institute (SBP)

Background: Fucosylation of proteins and lipids requires GDP-fucose. This donor is produced by either a *de novo* or a salvage pathway. The *de novo* pathway converts GDP-mannose to GDP-fucose accounting for >90% of cells' needs. The minor salvage pathway recycles intracellular L-fucose or imports extracellular L-fucose. It is converted to fucose-1p to then to GDP-Fucose. One of its primary uses is for core fucosylation of N-glycans using fucosyltransferase, FUT8.

Results: Using whole exome sequencing, we identified pathogenic mutations in two critical steps of fucosylation. The first involved three unrelated individuals with mutations in FUT8, encoding the only enzyme capable of N-glycan core fucosylation. Mass spectrometric analysis of serum samples from all three individuals revealed a complete loss of core fucosylation. Fibroblast were available from two cases and, using independent methods, we showed glycosylation abnormalities mirrored results from serum. Furthermore, fibroblast showed that both patient mutations resulted in no residual FUT8 protein.

The second disorder involves Fucokinase, which phosphorylates L-fucose to generate fucose-1p. When compared to three controls, patient fibroblast showed dramatically lowered levels of radioactively labeled L-fucose incorporation into fucosylated glycoproteins. These results were further replicated using a C13 stable isotope labeled L-fucose. Additionally, both fibroblast and lymphoblast had significantly reduced levels of FUK protein expression, suggesting the mutations affected protein stability. Consistent with the known minor contribution of the *de novo* pathway, there was no decrease of fucosylation in patient fibroblasts based on binding of fucose-specific lectins. Preliminary results of labeling cells with stable C13 fucose suggests that the *de novo* pathway may be expandable.

Conclusions: We present two novel types of Congenital Disorders of Glycosylation, FUT8-CDG and FUK-CDG that are due to deficiencies in cellular fucosylation.

Supported by: R01DK99551 and The Rocket Fund.

## **Exploring Treatment Approaches for PIGA-CDG**

**ANN NGUYEN**

PIGA Parent and Advocate

Phosphatidylinositol N-acetylglucosaminyltransferase subunit A (PIGA) is a protein subunit of an enzyme reaction involved in GPI-anchor biosynthesis. GPI-anchor plays a critical role in multiple biological processes including embryogenesis, neurogenesis, and immune response. PIGA mutation is classified as a congenital disorder of glycosylation (CDG) and causes a wide variety of symptoms including infantile spasms, profound intellectual disability, and often death.

Here we outline three novel treatment approaches identified through an in-depth review of the current scientific literature and understanding of the mechanism of disease. The basic principles can be applied to a broad range of CDGs and include 1) increasing the level of substrate for the enzyme reaction, 2) increasing expression levels of the mutated protein/enzyme, and 3) direct supplementation with the end product of the enzyme reaction.



Next, we summarize ongoing pre-clinical experiments and the current protocol being used to test the first two treatment approaches, N-acetylglucosamine and N-acetylcysteine, in a 2 year old male child with PIGA mutation.

Lastly, we outline the process and current progress towards manufacturing and testing the third identified drug treatment, N-acetylglucosylaminyl-Phosphatidylinositol (GlcNAc-PI), which includes finding a sponsoring IRB and identifying FDA requirements for conducting a single patient clinical trial.

## **Beyond ERAD: N-glycanase might bring you to tears**

**MITALI TAMBE, PH.D.**

Freeze Lab, Sanford Burnham Prebys Medical Discovery Institute (SBP)

Patients with mutations in NGLY1 cannot make tears (alacrima), have global developmental delay, movement disorder and liver dysfunction. N-glycanase 1 (NGLY1) de-glycosylates misfolded N-glycosylated proteins in the cytoplasm as part of the ERAD pathway prior to their proteasomal degradation. Surprisingly, NGLY1-deficient patient cells do not accumulate cytoplasmic misfolded N-glycoproteins, suggesting a more complex function.

Using WT and NGLY1-deficient mouse embryonic fibroblasts (MEFs) we found that NGLY1-deficient cells were resistant to hypotonic lysis compared to WT. The same was seen in NGLY1-patient fibroblasts. Water influx and cell swelling precedes cell lysis. We found that NGLY1-deficient MEFs swell slower than WT MEFs. Since aquaporins (AQP) transport water, we hypothesized that AQP levels might be disrupted in NGLY1-deficient cells. We found both AQP1 mRNA and protein were reduced in NGLY1-deficient MEFs. shRNA knockdown of AQP1 in WT MEFs decreased hypotonic lysis, suggesting AQP1 is associated with hypotonic lysis. NGLY1 shRNA and CRISPR studies confirmed that NGLY1 regulates AQP1 levels and hypotonic cell lysis. Preliminary studies show that complementing NGLY1-deficient MEFs with NGLY1 increases AQP1 and restores hypotonic lysis. Current efforts are directed towards understanding whether NGLY1 enzyme activity is necessary for AQP regulation.

We have identified a novel function of NGLY1, i.e. to directly/indirectly regulate AQPs. This finding may relate to NGLY1-deficient patients' inability to make tears. Future efforts will try to identify NGLY1-dependent transcription factors responsible for regulating AQPs. This work is supported by the Bertrand Might Research Fund and [NGLY1.org](http://NGLY1.org).

## **Long-term FKRP Gene Therapy is Effective in a Mouse Model of Muscular Dystrophy-Dyroglycanopathy**

**CHARLES H. VANNOY, PH.D.**

Research Scientist, McColl-Lockwood Laboratory for Muscular Dystrophy Research, Carolinas HealthCare System

Muscular dystrophy-dyroglycanopathies (MDGs) comprise a clinically and genetically heterogeneous group of rare and incurable muscle-wasting disorders characterized by defective glycosylation of  $\alpha$ -dyroglycan ( $\alpha$ -DG). To address the potential treatment of these types of disorders, our research team has focused on the preclinical development of a gene replacement therapy strategy utilizing an adeno-associated virus (AAV) vector expressing the fukutin-related protein (FKRP) gene, which plays a central



role in the post-translational modification of  $\alpha$ -DG. We have shown that systemic delivery of AAV-FKRP to a p.Pro448Leu mouse model of MDG can result in significant and sustained levels of FKRP in multiple tissues of interest and reduce the dystrophic pathology without any associated adverse effects. Results indicate that early treatment intervention coupled with a sufficient dose of AAV-FKRP can restore the biochemical defects with potential for improvement in the trajectory of disease progression and in expected lifespan. Additional results suggest that restoration of FKRP gene function may be able to halt disease progression at whatever stage it has reached, but has limited ability to reverse secondary pathologies. Nevertheless, limited benefits are achievable for advanced stages of the disease. These studies support the initiation of early-stage clinical trials for FKRP-related disorders.