• N-linked Glycoprotein Biogenesis and CDGs
• N-linked Glycans and Quality Control
• NGLY1 Deficiency
• Clinical Features of NGLY1 Deficiency (Lynne Wolfe)
• Mannose Oligosaccharide Glucosidase Deficiency (Sergio Rosenzweig)
NIH Glycosciences: A rich and lasting heritage

Claude Hudson
The founder of basic carbohydrate research at the NIH (Chief-1952)

Hewitt G. Fletcher
Chief, 1951-1973

G. Gilbert Ashwell
Discovery of Mammalian Lectins
Chief, LBM, 1978-1983

Elizabeth Neufeld
Chief, GBB 1979-1983

Victor Ginsburg
Chief, Lab Structural Biology
1986-1991

Roscoe Brady
NINDS
1972 to 2006

Glycoscience Interest Group
Undiagnosed Disease Program
Grace Wilsey was born with NGLY1 deficiency, which is caused by two mutations in the NGLY1 gene.
ONE OF A KIND
What do you do if your child has a condition that is new to science?

BY SETH MNOOKIN

In September of 2013, I visited the Mighels in Salt Lake City, where they have a home in a suburb of Salt Lake City, Utah. The Mighels have a daughter with a rare genetic disease, and her care was a major focus of our visit. I was struck by how devoted the Mighels are to her care and how much their lives revolve around her.

The Mighels are a family of three: Matt, Cristina, and their daughter, Bella. Bella was born in 2010 with a rare genetic disorder called the methylmalonic acidemia. The disease is caused by a mutation in a gene that codes for an enzyme called the methylmalonyl-CoA mutase. Without this enzyme, the body cannot break down certain fatty acids, leading to a build-up of toxic metabolites.

Bella's symptoms began to appear when she was just a few weeks old. She had difficulty breathing, and her parents noticed that she wasn't gaining weight. They took her to the hospital, and doctors found that her blood levels of the metabolite were extremely high. The Mighels were immediately referred to the Children's Hospital of Philadelphia, where they were able to get the best care possible.

At the hospital, the Mighels learned about the disease and about the treatments available. They were also able to connect with other families who had children with the same disease, and they were able to form a support group.

In July of 2014, I visited the Mighels again, this time at their home in Salt Lake City. They were celebrating Bella's birthday, and they had invited all of her friends to come over and celebrate. The Mighels were incredibly grateful for the support they had received from the support group, and they were happy to have the chance to celebrate with their daughter.

The Mighels have been able to make the most of their daughter's life. They have worked with the best doctors and therapists in the country, and they have been able to give Bella the best care possible. They have also been able to connect with other families who are going through the same thing, and they have been able to form a support group.

The Mighels are an amazing family, and they are doing an incredible job of taking care of their daughter. They are an inspiration to all of us who are trying to find a cure for this disease.
Glycans play a major role in human disease:

- Rarity/Severity of genetic diseases highlight the importance of glycans
- Some Examples of Glycans and Disease:
  - Defective O-glycosylation in Muscular Dystrophy
  - O-GlcNAcylation: Diabetes, Alzheimer’s, Cancer, Heart Disease.
  - Notch Signaling by Glycans
  - Selectins and Inflammation
  - Siglecs and Regulation of Immunity
  - Galectins role in immunity
  - Proteoglycans: growth factors, microbe binding, morphogenesis
  - Microbes and Viruses: Glycans role in entry and defense
  - Heparin – this ‘drug’ is a GAG.
  - Monoclonal Therapeutics – Glycoforms
  - Cell Surface Glycans in Tumor Metastasis – Cancer Biomarkers.
  - Vaccines to Infectious Organisms – Many (Most) are glycans.
N-Glycan Biosynthetic Pathway: A System to Generate Diversity.

What do we know?

1. Biochemistry
2. Inhibitors
3. Yeast and Somatic Cell genetics

How did we learn it?

- Biochemistry
- Inhibitors
- Yeast and Somatic Cell genetics
- Congenital Disorders of Glycosylation
CDG Disorders by Compartment

Mannose Pathway (3)

Cytosol

Sterol synthesis
Substrate and intermediate carrier

Dolichol Pathway (6)

ER (20)

ERGIC (1)

GOLGI (20)

Adapted from J Inherit Metab Dis (2011) 34:853-858
Thanks to Lynne Wolfe NP and Donna Krasnewich, MD, NGMS
The essential role of N-Glycosylation in ER Protein Folding
N-Glycans in Protein Folding Cycle and ERAD

Diagram showing the folding process, Calnexin cycle, and ERAD pathway. The process includes recognition, targeting, retro-translocation, ubiquitination, de-glycosylation, and degradation. Key components include Calnexin, BiP, Crt/Cnx, Grp94, EDEM1-3, Sel1, Derlin-1, Herp, VIMP, UBC7, p97-Ufd1-Npl4, AAA ATPase, and poly-Ub.
ONE OF A KIND

What do you do if your child has a condition that is new to science?

BY SETH MNOOKIN
Whole Exome Sequencing: Mutations in NGLY1 Cause an Inherited Disorder of the Endoplasmic Reticulum-Associated Degradation (ERAD) Pathway

Gregory M. Enns,…David Goldstein
Reaction scheme of PNGase.

PNGase (Ngly1)

Asn

Asp

Suzuki T J Biochem 2015;157:23-34
The involvement of cytoplasmic PNGase in ERAD. The glycoproteins destined for degradation are translocated from the ER lumen to the cytosol.
“We are fortunate that N-Glycanase was studied by the glycobiology community long before the discovery of the disorder.”

- N-Glycanase (encoded by the gene NGLY1) is responsible for cleaving N-linked glycans from misfolded glycoproteins, so that the body can recycle them.

- Lacking N-Glycanase leaves the body with an impaired capacity to recycle misfolded glycoproteins, which appear to accumulate in the cells of patients.

- The current hypothesis is that accumulation of these misfolded glycoproteins is what causes the harm in these patients.
Schematic representation of ENGase-mediated formation of N-GlcNAc proteins in Ngly1−/− cells.

Huang C et al. PNAS 2015;112:1398-1403
ROS generation by the ER and its interrelations to mitochondrial ROS generation.

Bashan N et al. Physiol Rev 2009;89:27-71
Clinical Features of NGLY1 deficiency

In addition to global developmental delay, neurological impairment, movement disorder and hypotonia, there are some symptoms that only appear in subgroups of the patient population. Each of the following symptoms has been found in at least half of all patients:

- **A lack of tears**

- **Liver dysfunction:** In particular, alpha-fetoprotein (AFP) may be extremely elevated while young.

- **A smaller head** (around the 5th percentile).

- **Diminished reflexes**

- **Material stored in liver cells:** There appears to be something stored in the cytoplasm of liver cells.

- **Seizures:** About half of all patients have observable seizures. Patient EEGs are often described as "abnormal."
Clinical application of exome sequencing in undiagnosed genetic conditions

Anna C Need,1 Vandana Shashi,2 Yuki Hitomi,1 Kelly Schoch,2 Kevin V Shianna,1 Marie T McDonald,2 Miriam H Meisler,3 David B Goldstein1,4

<table>
<thead>
<tr>
<th>Trio</th>
<th>Sex</th>
<th>Age</th>
<th>Race</th>
<th>Symptoms</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>M</td>
<td>3</td>
<td>European-American</td>
<td>Developmental delay, multifocal epilepsy, involuntary movements, abnormal liver function, absent tears</td>
</tr>
</tbody>
</table>

*J Med Genet 2012;49:353–361*
1st disorder of DE-Glycosylation

Mutations in NGLY1 cause an inherited disorder of the endoplasmic reticulum–associated degradation pathway

Gregory M. Enns, MB, ChB¹, Vandana Shashi, MD, MBBS², Matthew Bainbridge, PhD³, Michael J. Gambello, MD, PhD⁴, Farah R. Zahir, PhD⁵, Thomas Bast, MD⁶, Rebecca Crimian, MS², Kelly Schoch, MS², Julia Platt, MS¹, Rachel Cox, MS¹, Jonathan A. Bernstein, MD, PhD¹, Mena Scavina, DO⁷, Rhonda S. Walter, MD⁸, Audrey Bibb, MS⁴, Melanie Jones, PhD⁴, Madhuri Hegde, PhD⁴, Brett H. Graham, MD, PhD³, Anna C. Need, PhD⁹, Angelica Oviedo, MD¹⁰, Christian P. Schaaf, MD, PhD³,¹¹, Sean Boyle, PhD¹², Atul J. Butte, MD, PhD¹², Rong Chen, PhD¹², Michael J. Clark, PhD¹², Rajini Haraksingh, PhD¹², Tina M. Cowan, PhD¹³, FORGE Canada Consortium, Ping He, MD, PhD¹⁴, Sylvie Langlois, MD⁵, Huda Y. Zoghbi, MD³,¹¹,¹⁵, Michael Snyder, PhD¹², Richard Gibbs, PhD³, Hudson H. Freeze, PhD¹⁴ and David B. Goldstein, PhD¹⁶,¹⁷
Clinical Features of NGYL1

- Developmental delay (8/8)
- Movement disorder (8/8)
- Hypotonia (8/8)
- Alacrima/hypolacrimala (7/8)
- EEG abnormalities (7/8)
- Constipation (7/8)
- Transaminase elevation (6/7)
- Microcephaly (6/8)
- Decreased reflexes (6/8)
- Abnormal brain imaging (6/8)
- Abnormal liver storage (5/6)
- IUGR (5/8)
- Elevated blood lactate (4/6)
- Seizures (4/8)
- Strabismus (4/8)
- Corneal disease (4/8)
- Chalazions (4/8)
- Ocular apraxia (4/8)
- Neonatal jaundice (4/8)
- Dysmorphic features (4/8)
- Scoliosis (4/8)
- Small hands/feet (4/8)
- Peripheral neuropathy (3/3)
- Elevated AFP (3/5)
- ABR abnormalities (2/5)
- Liver fibrosis (2/6)

Found at NIH

Hypolacrima on Schirmer testing (8/8)
Optic nerve pallor (6/8)
Mild peripheral retinal pigmentary changes (5/8)
Near normal peripheral hearing sensitivity (8/9)
Hyperkinetic movement disorder (9/9)
Abnormal sweat response in a length dependent manner (5/8)
ABR Delayed and/or dysynchronous transmission through the brainstem (7/8)
History of absence/atonic/myoclonic seizures (5/9)
Delayed bone age (6/8)
Developmental delay (9/9)
Demyelinating Axonal sensorimotor polyneuropathy (5/8)
Mostly resolved transaminitis (6/9 (one liver transplant))
Abnormal liver texture on ultrasound (5/9)
Cerebral atrophy (3/8)
Clinical Features of NGYL1

NEW Findings from NIH
Abnormal neurotransmitter levels (3/8)
Low CSF protein (6/8) and albumin levels (8/8)
Hyper immune response to the rubella and rubeola vaccination (7/8),
Lower than predicted resting energy expenditure (8/8)
Consistently affectionate and happy demeanor (8/8)

PERTINENT NEGATIVES from NIH
Normal echocardiogram
Normal gastric fluid pH
No evidence of primary muscle disease on EMG
No evidence of aspiration by Swallow study
NGLY1-CDG
NIH