Hematopoietic stem cell transplantation to correct neutropenia and lymphopenia in a new type of CDG

presented by

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Collaborative project:
USA – Norway - Germany - Japan
All three patients shared the immunophenotype: T- B- NK+ SCID and neutropenia.

Patient 1
Skeletal phenotype

Patient 2
No skeletal phenotype

Patient 3
Skeletal phenotype

Published AJHG, 2014, Stray-Pedersen A. et al
Severe immunodeficiency, neutropenia, and skeletal dysplasia

Patient 1  Afghan

Patient 3  German

Similar to Desbuquois dysplasia (DBQD), extra ossification center, monkey wrench femora
Severe immunodeficiency, neutropenia, +/- skeletal dysplasia

Patient 2: Normal growth, no skeletal dysplasia, no dysmorphic features, and normal development intellectually.

[Graph showing growth and weight trends with data points marked by crosses.]

Stature (cm) vs. Age in years

Weight (kg) vs. Age in years
Clinical features

- eczema, abscesses, recurrent infections,
- Severe immune deficiency, T- B- NK+ SCID
- Congenital neutropenia
- Progression to bone marrow failure
- +/- developmental delay
- +/- severe skeletal dysplasia DBQD
Hematopoietic stem cell Transplantation (HSCT)

2 of 3 cured, 1 died before HSCT

T^- B^- NK^+ SCID
Severe Combined Immunodeficiency

Neutropenia

Neutrophil, Eosinophil, Monocyte, Macrophage

Erythrocyte, Mast cell, Myeloblast, Natural killer cell (Large granular lymphocyte), Small lymphocyte, T lymphocyte, B lymphocyte, Plasma cell

Megakaryocyte

Thrombocytes
Hematopoietic stem cell transplantation, HSCT

**Donor**
- Bone marrow blood or umbilical cord blood

**Recipient**
- Precoditioning with cytostatics
- Healthy immune system
Results from whole exome sequencing:

**PGM3, phosphoglucomutase 3**

**Mutations in PGM3 cause a novel glycosylation defect, PGM-CDG**

All 3 patients had one missense mutation...residual enzyme activity?
PGM3 protein structure:

- Binds substrate
- Affects active site
PGM3 enzyme,
- a step towards UDP-GlcNAc

Glucose

\[
\text{N-acetylglucosamine 6-phosphate} \leftrightarrow \text{N-acetylglucosamine 1-phosphate} \leftrightarrow 
\]

PGM3

\[
\text{UDP-N-acetyl-glucosamine}
\]

Freeze H H (2013) J. Biol. Chem.;
Freeze H H and Alan D Elbein A D. (2009)
Essentials of Glycobiology
PGM3 enzyme, - a step towards UDP-GlcNAc

Glycosylation = sugar branching = modification of proteins and lipids

2 linkage types:
• N-glycosylation
• O-glycosylation

1 out of 9 activated sugars

PGM3 enzyme,
- a step towards UDP-GlcNAc

Glucose

N-acetylglucosamine 6-phosphate

N-acetylglucosamine 1-phosphate

PGM3

UDP-N-acetyl-glucosamine (UDP-GlcNAc)

- activated sugar in **both** N-linked and O-linked protein glycosylation
- incorporated into N-glycans, O-glycans and glycosylphosphatidylinositol (GPI)-anchored proteins
- donor for reversible addition of O-GlcNAc to proteins
- proteoglycan synthesis

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*Essentials of Glycobiology*
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Essentials of Glycobiology
PGM3 enzyme,
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Glucose

**PGM3**

N-acetylglucosamine

6-phosphate

N-acetylglucosamine

1-phosphate

UTP

PPᵢ

*UDP-N-acetyl-glucosamine*

**Mouse Model:**
- *Pgm3*-/ embryonic lethal
- *Pgm3* hypomorphic:
  - tri-lineage cytopenias
  - growth retarded

**our patients:**

Serum samples tested in diagnostic lab pre- and post HSCT:
- normal N-glycosylation Transferrin CZE
- normal O-glycosylation. APOCIII

**E. coli** mutant models

- Asn246Ser (Patient 1): 1% enzyme activity
- Asp239His (Patient 2): 59% enzyme activity
- Gln451Arg (Patient 3): 50% enzyme activity

*Patient 1: baseline/post HSCT sampling
Patient 2: post HSCT sampling
Patient 3: baseline sampling

PGM3-CDG - a broad and variable phenotype

eczema, abscesses, recurrent infections, developmental delay

T- B- NK+ SCID, Severe Congenital Neutropenia, Bone Marrow Failure, Desbuquois dysplasia

Hyper IgE, Neutropenia
Bronchiectasis +/- scoliosis
CD8 lymphopenia, or CD4 lymphopenia. High cholesterol levels, Renal and liver changes

Patient 1  Patient 3
Patient 2

Sassi et al: 9 affected, 4 families
Zhang et al: 8 affected, 2 families

Asp239His (Patient 2) (het + LOF)
Asp325Glu (hom)
Gln451Arg (Patient 3) (het+LOF)
Glu529Gln (het + LOF)

1) Zhang et al. (May 2014) JACI
2) Sassi et al. (May 2014) JACI
3) Stray-Pedersen et al. (July 2014) AJHG
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Reduced PGM3 activity

-> Reduced **UDP-GlcNAc** levels

-> Reduced glycosylation branching

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1) Zhang et al. (May 2014) JACI
2) Sassi et al. (May 2014) JACI
3) Stray-Pedersen et al. (July 2014) AJHG
4) Yang et al. (Dec 2014) Curr Opin Pediatr.
Glycosylation Disorders
with defect ‘sugar activation’

Figure modified from
Glycosylation Disorders
with defect ‘sugar activation’

Glucose
ATP

Glc-6-PO_4

Glu-6-PO_4

Glutamine

GFAT
Glutamate

NH_3

Glucosamine-6-PO_4

AcetylCoA

CoA

GlcNAc-6-PO_4

PGM3

GlcNAc-1-PO_4

UTP

PP_i

UDP-GlcNAc

Activated sugars

Figure modified from Freeze H H (2013) J. Biol. Chem.
Glycosylation Disorders
with defect ‘sugar activation’

Activated sugars

Figure modified from Freeze H H (2013) J. Biol. Chem.
Glycosylation Disorders with defect 'sugar activation'

Activated sugars

Figure modified from Freeze H H (2013) J. Biol. Chem.
Glycosylation Disorders with defect ‘sugar activation’

Control Points
Activated sugars
Peroral Treatment

Figure modified from Freeze H H (2013) J. Biol. Chem.
Glycosylation Disorders with defect ‘sugar activation’

Activated sugars

Peroral Treatment
Glycosylation Disorders with defect ‘sugar activation’

Activated sugars
Peroral Treatment?

Figure modified from Freeze H H (2013) J. Biol. Chem.
**Glycosylation Disorders with defect ‘sugar activation’**

**Activated sugars**

**Peroral Treatment?**

Figure modified from Freeze H H (2013) J. Biol. Chem.
**Immunologic Effects of Supplemental Monosaccharide and Nucleoside Derivatives in Patients With Inherited Disorders of Glycosylation**

**This study is currently recruiting participants. (see Contacts and Locations)**

Verified May 2015 by National Institutes of Health Clinical Center (CC)

**Sponsor:**
National Institute of Allergy and Infectious Diseases (NIAID)

**Information provided by (Responsible Party):**
National Institutes of Health Clinical Center (CC) (National Institute of Allergy and Infectious Diseases (NIAID))

**ClinicalTrials.gov Identifier:**
NCT02511041

First received: July 26, 2015
Last updated: August 19, 2015
Last verified: May 2015

**Purpose**

Clinical trial started 2015: N-Acetylglucosamine (GlcNAc) oral supplement

**Background:**
- A congenital disorder of glycosylation (CDG) affects the cells that make up the organs and tissues. In these cells, sugar molecules do not properly attach to other molecules, which are the basic building blocks of cells. Changes in sugars seen in people with CDGs may lead to allergies and can change people's ability to fight infections. Researchers want to see if a sugar supplement called N-acetylglucosamine can help people with CDGs who have detectable changes in their immune systems.

**Objective:**
- To see if N-acetylglucosamine can help cells to function in a healthy way in people with CDGs.

**Eligibility:**
- People at least 2 years of age who have a CDG and immune system changes.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intervention</th>
<th>Phase</th>
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<tbody>
<tr>
<td>PGM3</td>
<td>Drug: N-Acetylglucosamine (GlcNAc)</td>
<td>Phase 1</td>
</tr>
<tr>
<td></td>
<td>Drug: Uridine</td>
<td></td>
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</tbody>
</table>
How to diagnose patients with CDG-PGM3?

- Not detectable on routine glycosylation tests
- Awareness of the various clinical features
- Genetic testing; Whole exome sequencing
- May be detected on new newborn screening
produce an1bodies

T-B-NK+ SCID
Severe Combined Immunodeficiency

Neutropenia

T lymphocyte

Multipotential hematopoietic stem cell
(Hemocytoblast)

Common myeloid progenitor

Erythrocyte

Mast cell

Myeloblast

Common lymphoid progenitor

Natural killer cell

(Large granular lymphocyte)

Small lymphocyte

B lymphocyte

Plasma cell

produce antibodies

Megakaryocyte

Thrombocytes

Basophil

Neutrophil

Eosinophil

Monocyte

Macrophage
T lymphocyte development

To create T cell specificity, the T cell receptor DNA is rearranged:

- Circular DNA
- Can be measured in newborns
- No/low TRECs = SCID

Severe combined immunodeficiency

- NEW NEWBORN SCREENING
  Will detect PGM3-CDG-SCID!

T-lymphocyte development

- Stem cell
- Lymphoid progenitor
- Double negative 1, 2, 3
- Immature single positive
- Double positive
- Single positive
- Blood
- CD4
- CD8

Final T cell receptor code

T-cell exision circles, TRECs

Tribute to Prof. Jennifer Puck who invented SCID screening using TRECs

Patient 1, 1.5 years after her hematopoietic stem cell transplantation: Progression of skeletal dysplasia and the scoliosis.

And also Patient 2 has developed scoliosis 2 y post-HSCT.
Patient 1, 1.5 years after HSCT:
Developmental delay.
No progression of brain abnormalities on MRI scan.

Adult patients with PGM3-CDG, reported by Zhang et al: Dysmyelination on MRI.
Conclusions

PGM3-CDG

• Recognizable phenotype:
  – Immunodeficiency
  – Skeletal
  – Broad/variable phenotype

• Genotype-phenotype correlation may exist:
  – Glycosylation branching

• Routine screening for N- and O- glycosylation: Normal results
  – Only genetic testing reveals the diagnosis, or TREC screening

• Treatment:
  – Hematopoietic stem cell transplantation cures immune defect
  – HSCT does not cure the skeletal dysplasia.
  – Peroral treatment trials - aiming to increase UDP-GlcNAc

Similar to Galactose in PGM1 deficiency and Mannose in MPI deficiency
Acknowledgements

Baylor College of Medicine & Texas Children’s Hospital
Houston, TX
Tomek Gambin
Niti Choksi
Ghadir S. Sasa
Robert A. Krance
Caridad A. Martinez
Lisa Forbes
Shalini Jhangiani
Donna M. Muzny
Ankita Patel
Christine R. Beck
Eric Boerwinkle
Richard A. Gibbs
Jordan S. Orange
I. Celine Hanson
James R. Lupski

Baylor-Hopkins Center for Mendelian Genomics

Freiburg University Hospital
Freiburg, Germany
Marcin Wlodarski
Marcus Krüger
Carsten Speckmann
Stephan Ehl
Ekkehart Lausch

Emory University
Decatur, GA
Patricia Hall

Mayo College of Medicine
Rochester, MN
Kimiyo Raymond

University of New Mexico
Albuquerque, NM
Shirley M. Abraham
Mary Martinez

Oslo University Hospital
Norway
Hanne S. Sorte
Paul H. Backe
Lars Mørkrid
Hans Christian Erichsen
Katja B. P. Elgstøen
Magnar Bjørås
Else Merckoll
Jostein Westvik
Cecilie Rustad
Tore G. Abrahamsen
Arild Rønnestad
Liv T. Osnes
Torstein Egeland
Olaug K. Rodningen

University of Tokyo
Japan
Gen Nishimura

Special appreciation to the patients and their families for allowing this presentation of their unique disorder

Asbjørg Stray-Pedersen, MD PhD, Norwegian National Unit for Newborn Screening