The clinical phenotype of \textit{PIGN} deficiency and consequences of defective GPI biogenesis

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World Glycobiology Congress
Philadelphia, PA
Introduction

• Congenital (genetic) disorders of glycosylation (CDG) are a rapidly growing disease family

• Approximately 100 disorders have been identified
Introduction

• Glycosylation related genes are thought to comprise approximately 1–2% of the human genome.

• A new glycosylation disorder was reported, on average, every 17 days in 2013, in large part due to extensive sequencing of patient exomes and genomes.
Introduction

- Most CDGs are protein hypoglycosylation disorders, with defects in:
  - *N*-glycosylation pathway
  - *O*-glycosylation pathway
  - both the *N*- and the *O*-glycosylation pathways
Disorders also occur in the synthesis of lipid-based pathways
GPI biogenesis defects are a subtype of the congenital disorders of glycosylation.

These disorders involve the intersection of two pathways: lipids and carbohydrates.
The Glycosylphosphatidylinositol (GPI) anchor
GPI anchoring

- Glycosylphosphatidylinositol (GPI) anchoring of proteins is a highly conserved process present in most eukaryotic cells.

- GPI-anchored proteins perform a diverse set of functions including roles in signal transduction, cell adhesion and antigen presentation.
GPI anchored proteins

CD59
Alkaline phosphatase
Cell surface hydrolases (many)
Neural cell adhesion molecule 120 (NCAM-120)
Neural cell adhesion molecule TAG-1
The GPI pathway
Defective GPI biosynthesis

12 genes: PIGA, PIGM, PIGN, PIGV, PIGL, PIGO, PIGT, PGAP2, PIGW, PGAP1, PGAP3, ST2GAL5

Mutations are associated human disorders

• Various congenital anomalies
• Epilepsy/seizures
• Developmental delay/Intellectual disabilities
GPI ethanolamine phosphate transferase 1
First family described by Maydan et al in 2011

Mapped the disease locus

18q21.32e18q22.1

identified the disease-causing mutation

c.2126G/A (p.Arg709Gln)

in the **PIGN** gene

Multiple congenital anomalies-hypotonia-seizures syndrome is caused by a mutation in **PIGN**


Multiple congenital anomalies-hypotonia-seizures syndrome 1 (OMIM#614080)

Described a Autosomal recessive syndrome:

- Developmental delay
- Dysmorphic features
- Multiple congenital anomalies involving the cardiac, genitourinary and gastrointestinal systems
- Severe neurological impairment with chorea and seizures leading to early death

**PIGN** mutations cause congenital anomalies, developmental delay, hypotonia, epilepsy, and progressive cerebellar atrophy

Two Japanese Siblings

Developmental delay
Hypotonia
Seizures
Nystagmus
Tremors
Abnormal facial features
Abnormal Brain MRI with delayed myelination and cerebellar atrophy

Ohba et al (2014)
Exome report

Exome sequencing identifies a recessive *PIGN* splice site mutation as a cause of syndromic Congenital Diaphragmatic Hernia

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Identified a homozygous splice site mutation in the *PIGN* gene in a fetus with multiple congenital anomalies including bilateral diaphragmatic hernia, cardiovascular anomalies, segmental renal dysplasia, facial dysmorphism, cleft palate, and oligodactyly

*European Journal of Medical Genetics 57 (2014) 487e493*
Cystic hygroma
Cleft palate
Small penis with hypospadias

Broad nose
Hypertelorism, anteversion of the nostrils
Low set dysplastic ears

Left foot with oligodactyly
Synovial cyst attached to the left heel

Brady et al (2014)
2 y.o male

Severe hypotonia
Genital anomalies
Visual impairment
Supernumerary nipples
Pectus excavatum
Dysmorphic features
OUR CASES
Patient 1

3 months old girl:

- **Seizures** since first few days of life, intractable
- **Hypotonia**
- **Multiple congenital anomalies**
Dysmorphic features:

Mild proptosis
Hypertelorism
Thickened helices with posteriorly rotated ear
Tented upper lip
Small chin
Patient 1

Short fingers with hypoplastic distal phalanges

Bilateral fifth finger clinodactyly
Clinical course:
Extensive Metabolic and genetic testing including WES

Progressed to chronic encephalopathy with intractable seizures
Parents opted for DNR
Patient passed away at 6 months from respiratory failure with febrile illness
Patient 1 diagnosis

Whole Exome Sequencing (WES)
Whole Exome Sequencing (WES)

Compound heterozygous mutations in *PIGN*

c.1674+G>C (IVS18+1 G>C)

c.2679 C>G (p.S893R)
Patient 2

- Hypotonia
- Seizures
- Multiple congenital anomalies
Patient 2

- Large anterior fontanel bitemporal narrowing
- Wrinkled skin around the eyes
- Ears with thickened, over folded helix
- Tented lip
Patient 2

- Hypoplastic nails, absent nail on 5th toes
- Dermatoglyphics 8/10 arches
Patient 2

- PDA on echo
- Small splenic cyst on abdominal US
- Imperforate anus with perineal fistula
- Uterine didelphys
Patient 2 Diagnosis

Compound heterozygous mutations in *PIGN*

c.2126G->A (p.Arg709Gln)
c.287C>G (p.Pro96Arg)
Patient 3

Milder phenotype
• Early onset refractory epilepsy
• Hypotonia
• Developmental delay
• Minor dysmorphic features
Patient 3

Dysmorphic features:

- Uplslanting parbebral fissures
- Long philtrum
- Thin upper lip
- Broad nasal tip
- Thickened helix
Patient 3

- Compound heterozygous mutations in PIGN
  - c.932T>A (p.L311W)
  - c.806-4_808del GTAGGTT
What are we interested in?
Our research

- Clinical characterization/phenotype
- Biomarker
- Insights of pathogenesis
- Potential therapies
It has only been within the last few years that the majority of human disorders have been identified thanks to advances in NGS.

Prior to this the only deficiency within GPI biosynthetic pathway was caused by somatic mutations in PIGA.
The Pathobiology of PNH

- PIG-A gene is one of >20 genes involved in GPI formation.
- A somatic mutation in the PIG-A gene prevents all GPI-anchored proteins from binding to cell surface.

**CD59**
- Forms a defensive shield for RBCs from complement-mediated lysis
- Inhibits the assembly of the membrane attack complex

**CD55**
- Prevents formation and augments instability of the C3 convertases, attenuating the complement cascade

Adapted from Brodsky R. Paroxysmal Nocturnal Hemoglobinuria. 2005;419-427.[2]
One limitation in the identification of cases related to GPI deficiencies is the lack of facile and clear biomarkers.

Nearly all the deficiencies have been identified or solved by NGS and not by screening biomarkers.
• Maydan et al 2011-> CD59 in fibroblasts

• Chihiro et al 2014-> CD16, CD24 in granulocytes and LCL’s

• Krawtiz et al 2010-> FLAER, CD16 in leukocytes of patients with PIGV mutations
PIGN Project

• Develop a FACS protocol to prove GPI anchor deficiency

• Examine the functional impairment of PIGN caused by compound heterozygous mutations by analyzing the surface expression of various GPI-Aps Fluorescence activated cell sorting
GPI anchored surface markers

- **CD59** → Complement mediation
- **CD90** → Cell-cell interaction
- **CD16** → Fc receptor, immune system
FACS analysis

Patient 2

Patient 3

Normal human fibroblasts

CD59 negative (unstained)
FACS analysis

Patient 2

Patient 3

Normal human fibroblasts

CD59 negative (unstained)
Conclusions

- Patients with *PIGN* mutations can present with variable clinical phenotypes affecting different body systems.
- Consistent features observed include hypotonia, seizures, developmentally delay and facial dysmorphism.
- FACS analysis of GPI anchored proteins on affected patients fibroblasts can help prove deficient GPI anchor biogenesis.
Future directions

Functional studies are currently underway in our Lab at CHOP to further characterize the consequences of these mutations.
Thank you!

Your questions, comments and Ideas are appreciated