The clinical phenotype of *PIGN* deficiency and consequences of defective GPI biogenesis

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World Glycobiology Congress
Philadelphia, PA

Children's Hospital of Philadelphia

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

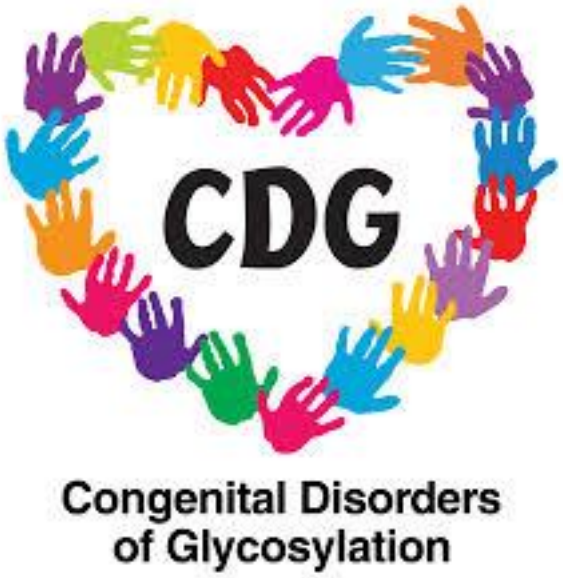
- Most CDGs are protein hypoglycosylation disorders, with defects in:

- *N*-glycosylation pathway
- *O*-glycosylation pathway
- both the *N* - and the *O*-glycosylation pathways

GPI anchor biogenesis defects

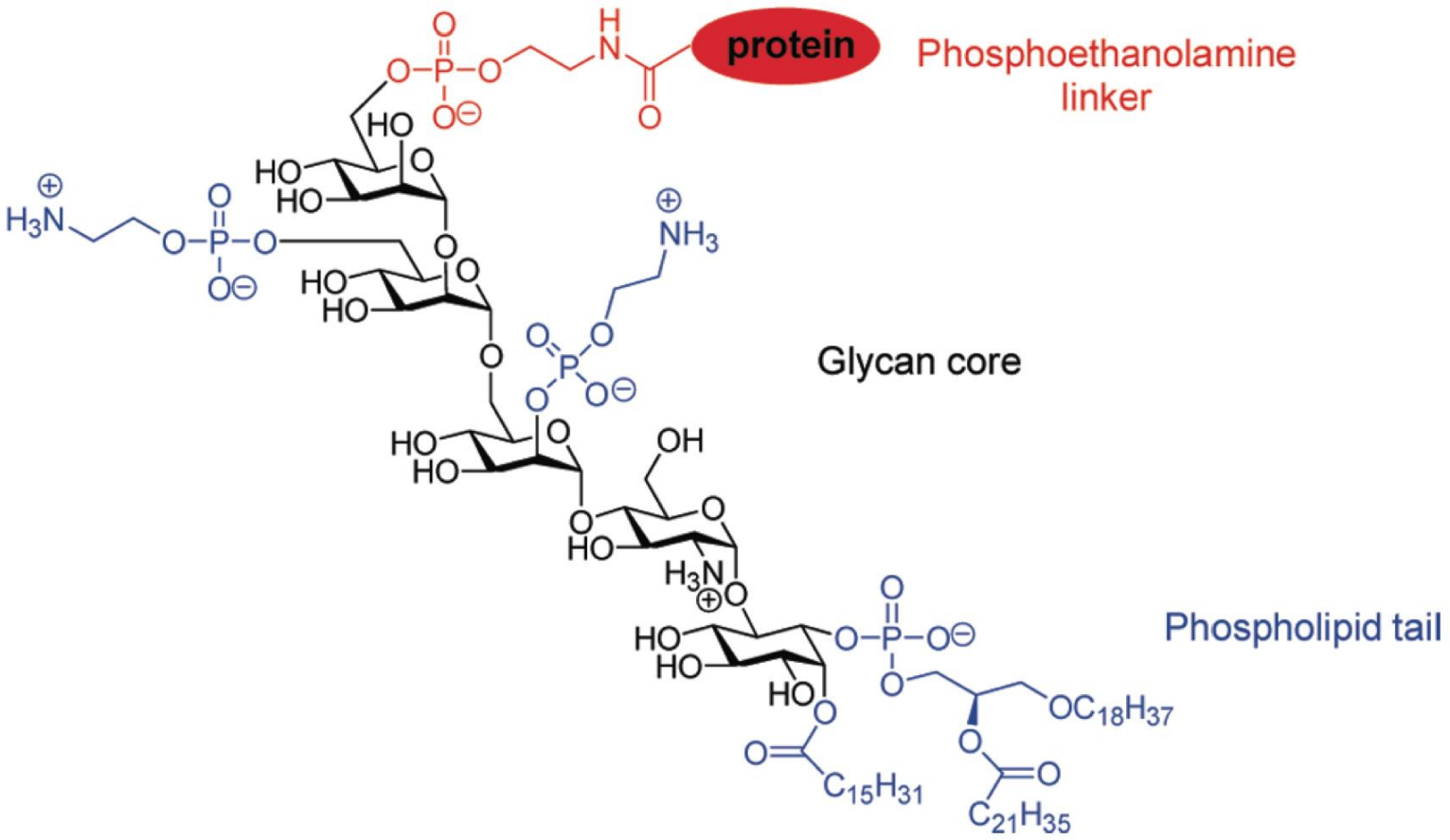
Disorders also occur in the synthesis of lipid-based pathways

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

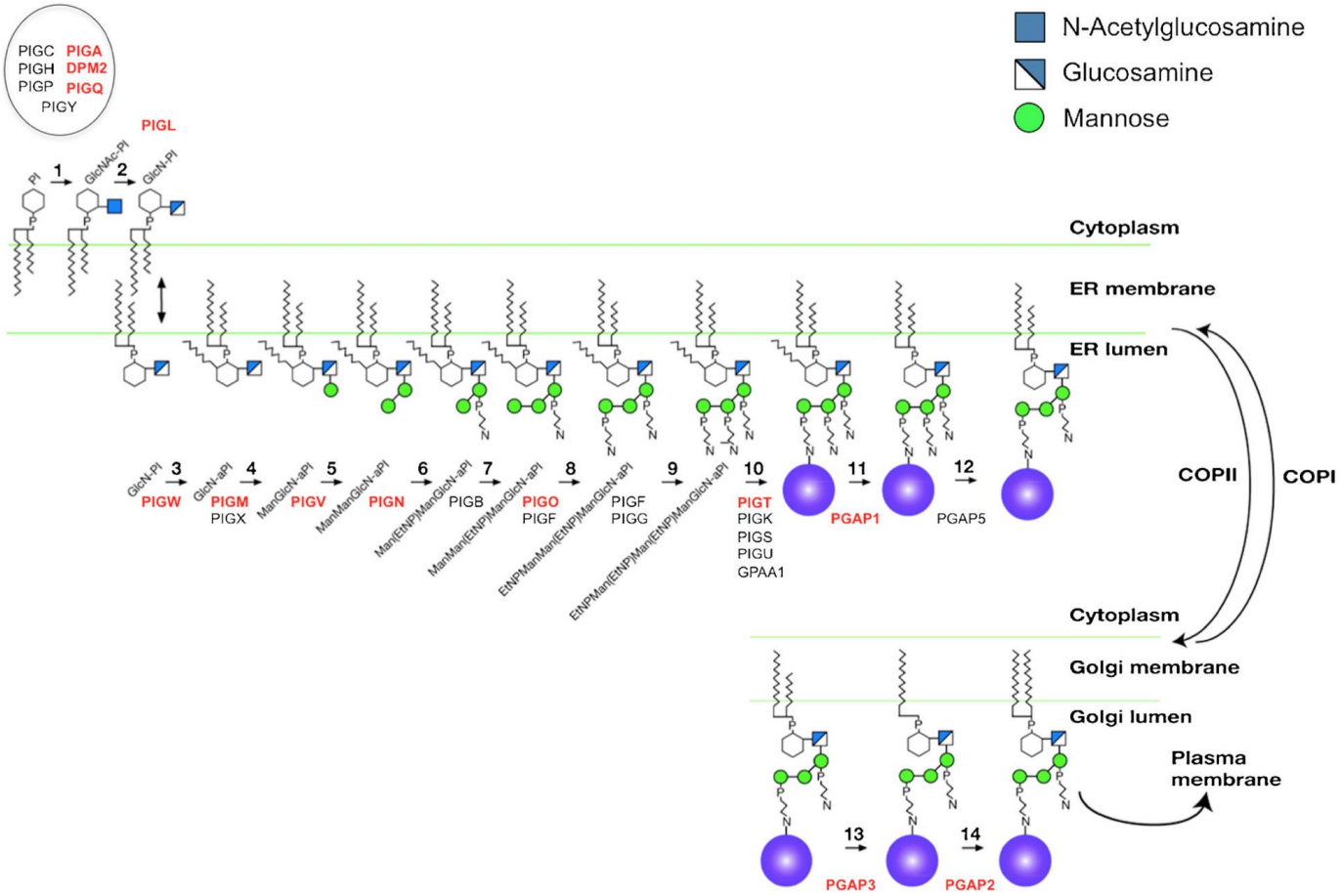
Alkaline phosphatase

Cell surface hydrolases (many)

Neural cell adhesion molecule 120 (NCAM-120)

Neural cell adhesion molecule TAG-1

The GPI pathway



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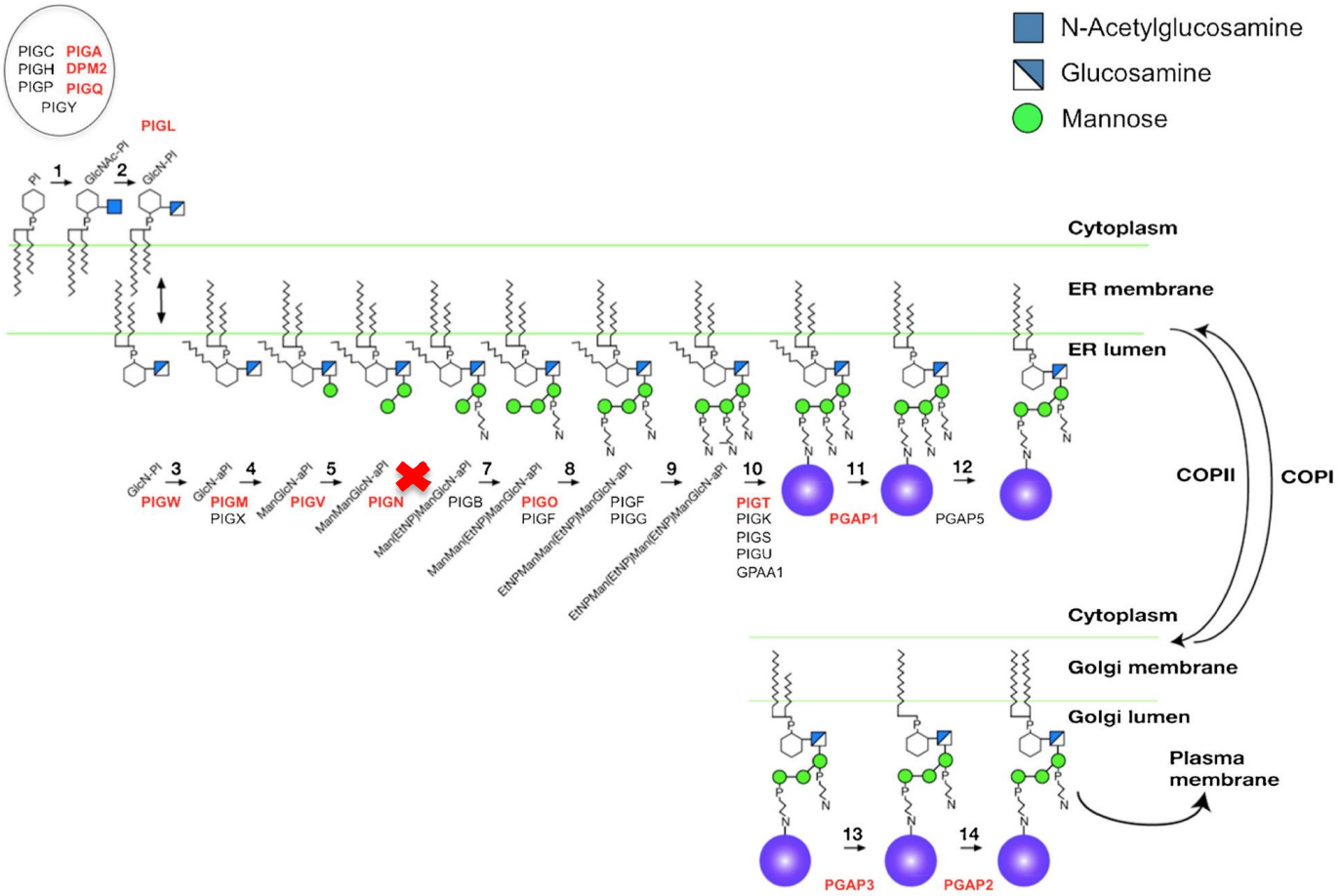
Defective GPI biosynthesis

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

The GPI pathway



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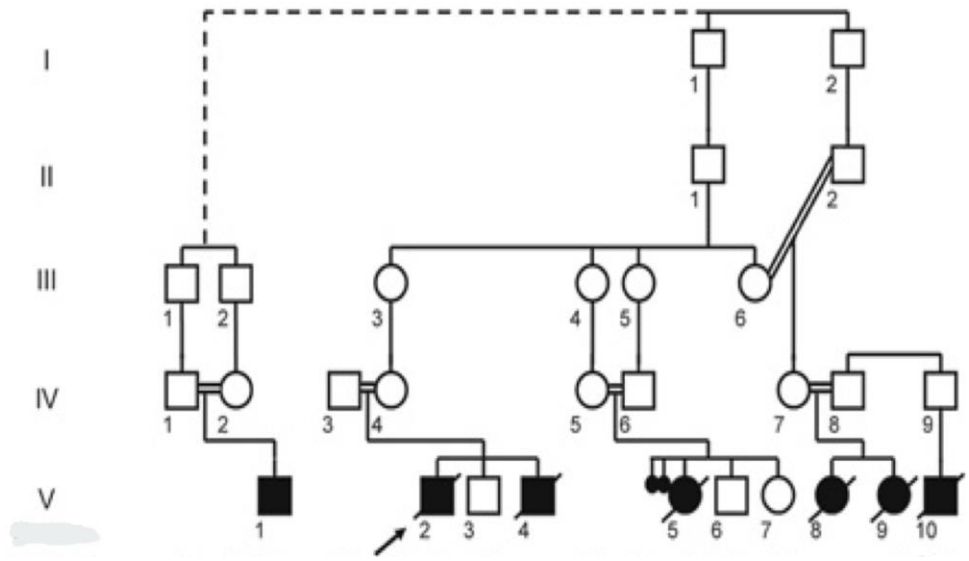
GPI ethanolamine phosphate transferase 1

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*

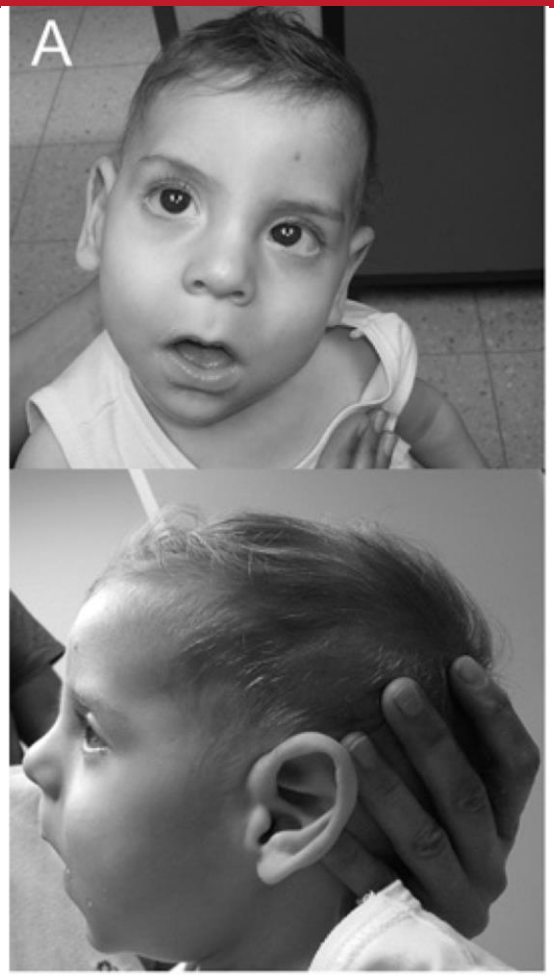


Multiple congenital anomalies-hypotonia-seizures syndrome is caused by a mutation in *PIGN*

Gal Maydan,[1] Iris Noyman,[2] Adi Har-Zahav,[3] Ziva Ben Neriah,[4] Metsada Pasmanik-Chor,[5] Adva Yeheskel,[5] Adi Albin-Kaplanski,[6] Idit Maya,[7] Nurit Magal,[7] Efrat Birk,[3] Amos J Simon,[8] Ayelet Halevy,[2] Gideon Rechavi,[3,8] Mordechai Shohat,[3,7,9] Rachel Straussberg,[2,3] Lina Basel-Vanagaite[3,7,10]

J Med Genet 2011;48:383e389.

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

Maydan et al J Med Genet 2011;48:383e389

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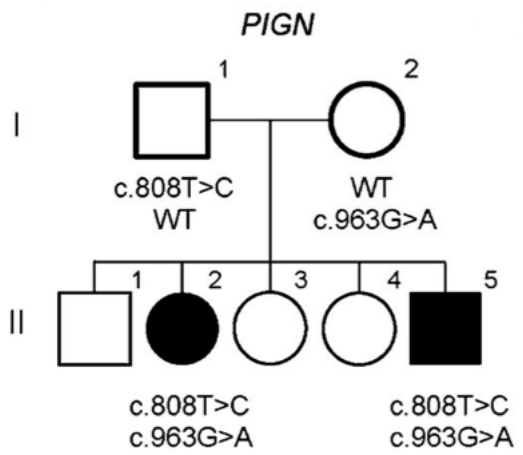
Ohba et al (2014)

Neurogenetics (2014) 15:85–92
DOI 10.1007/s10048-013-0384-7

ORIGINAL ARTICLE

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

Chihiro Ohba · Nobuhiko Okamoto · Yoshiko Murakami · Yasuhiro Suzuki · Yoshinori Tsurusaki · Mitsuko Nakashima · Noriko Miyake · Fumiaki Tanaka · Taroh Kinoshita · Naomichi Matsumoto · Hirotomo Saitsu



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Ohba et al (2014)

Two Japanese Siblings

Developmental delay

Hypotonia

Seizures

Nystagmus

Tremors

Abnormal facial features

Abnormal Brain MRI with delayed myelination and **cerebellar atrophy**

Brady et al (2014)

Exome report

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

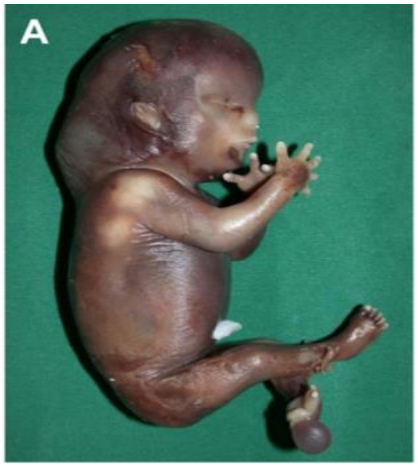
P.D. Brady [a], Philippe Moerman [b], Luc De Catte [c,d], J. Deprest [c,d], K. Devriendt [a], J.R. Vermeesch [a,*]

[a] *Centre for Human Genetics, KU Leuven, University Hospital Leuven, Belgium*
[b] *Translational Cell & Tissue Research Unit, Department of Imaging and Pathology, KU Leuven, University Hospital Leuven, Belgium*
[c] *Department of Development and Regeneration, Unit Pregnancy, Foetus and Newborn, KU Leuven, University Hospital Leuven, Belgium*
[d] *Department Obstetrics and Gynaecology, University Hospital Leuven, Belgium*

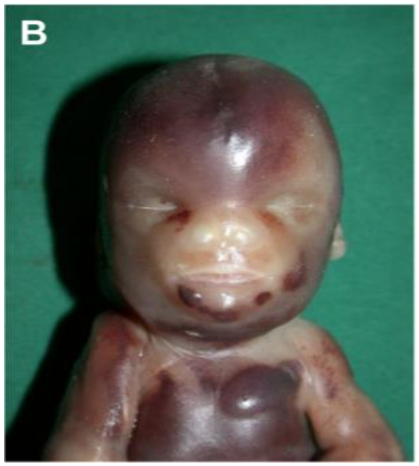
Identified a homozygous splice site mutation in the *PIGN* gene in a fetus with multiple congenital anomalies including bilateral diaphragmatic hernia, cardiovascular anomalies, segmental renal dysplasia, facial dysmorphism, cleft palate, and oligodactyly

European Journal of Medical Genetics 57 (2014) 487e493

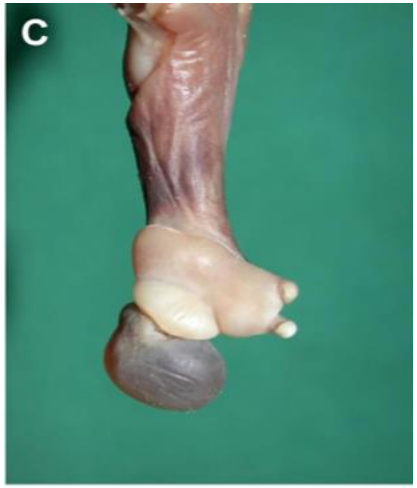
Brady et al (2014)



Cystic hygroma
Cleft palate
Small penis with hypospadias



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

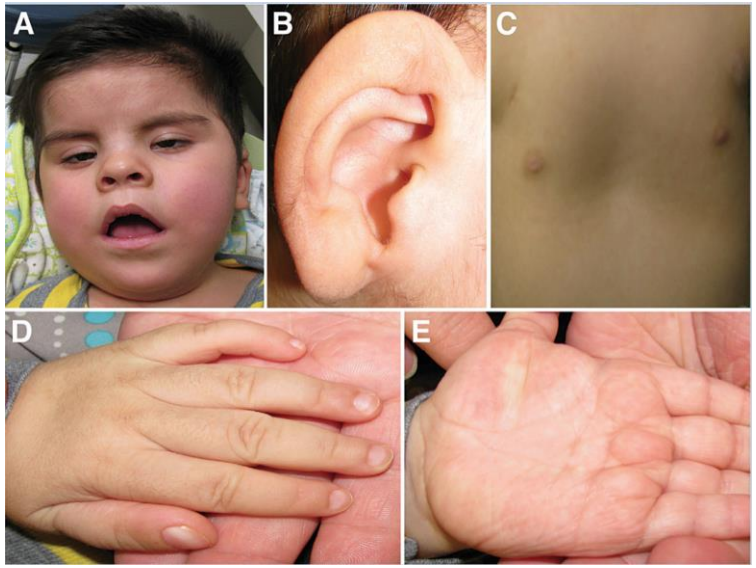


Left foot with oligodactyly

Synovial cyst attached to the left heel

Couser et al (2015)

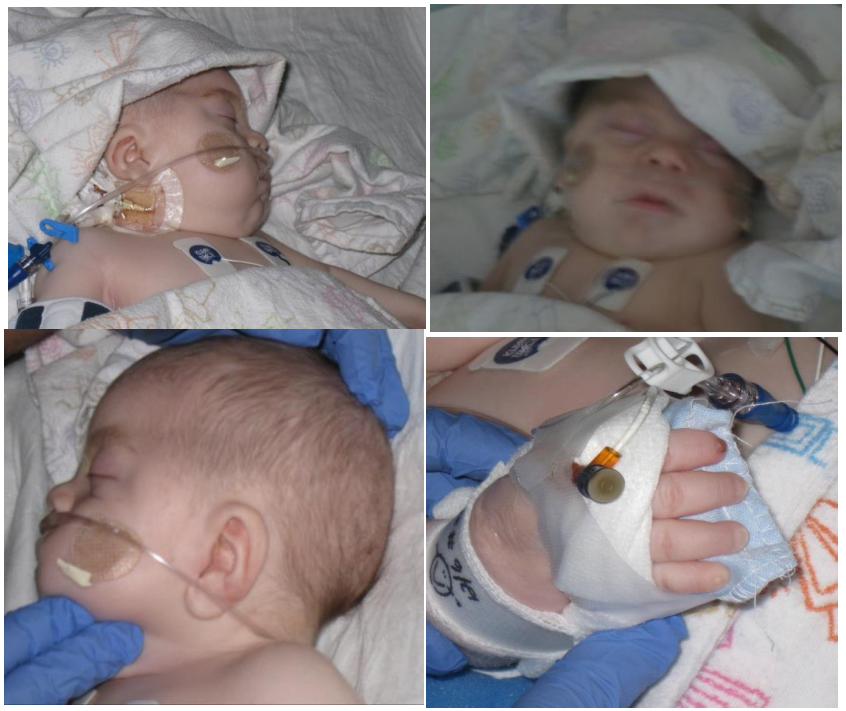
2 y.o male



Severe hypotonia
Genital anomalies
Visual impairment
Supernumerary nipples
Pectus excavatum
Dysmorphic features

OUR CASES

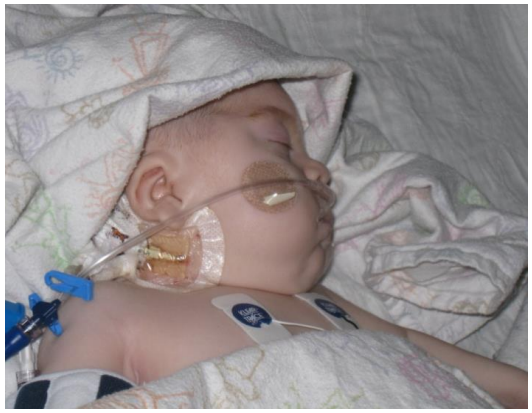
Patient 1

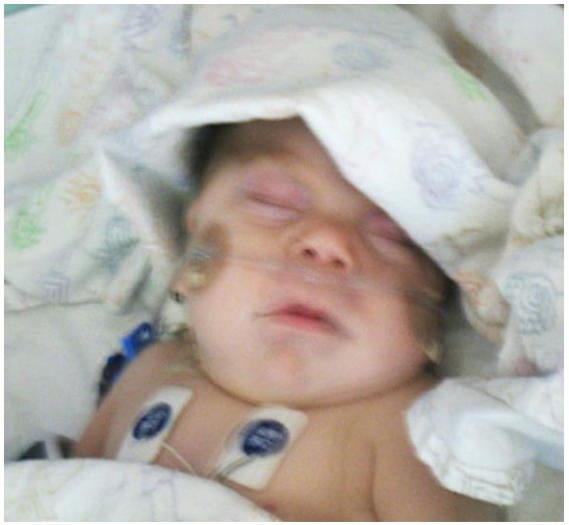


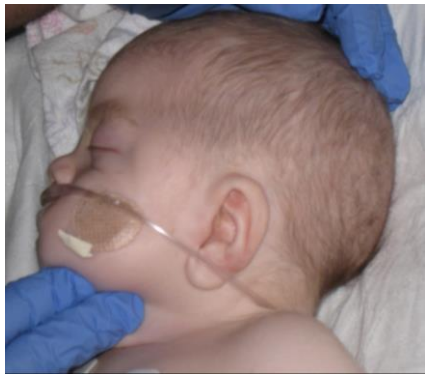
3 months old girl:

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

Patient 1







Dysmorphic features:

Mild proptosis

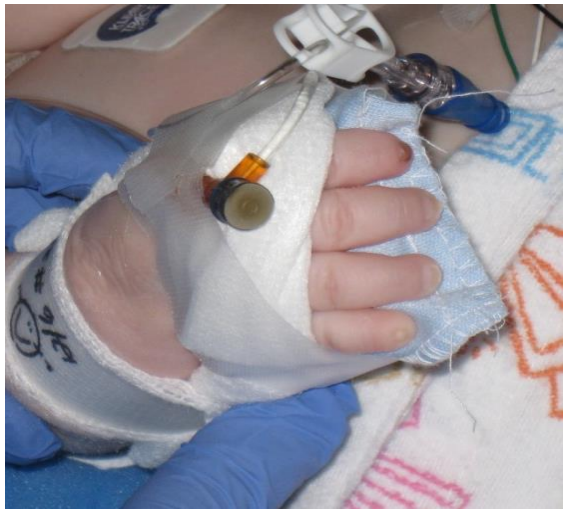
Hypertelorism

Thickened helices with posteriorly rotated R ear

Tented upper lip

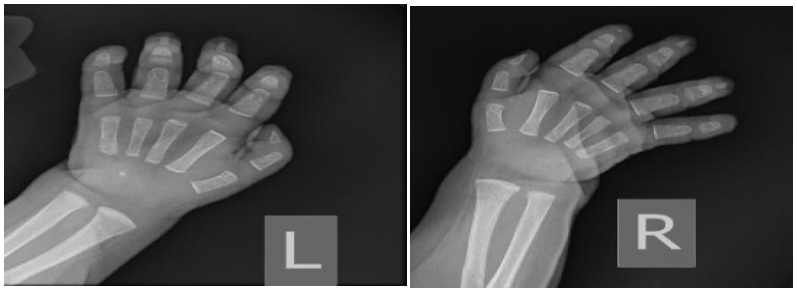
Small chin

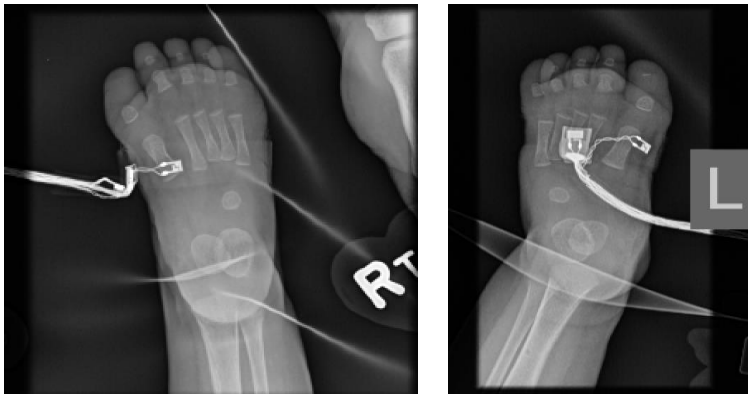
Patient 1



Short fingers with hypoplastic distal phalanges

Bilateral fifth finger clinodactyly





Patient 1

Clinical course:

Extensive Metabolic and genetic testing including WES

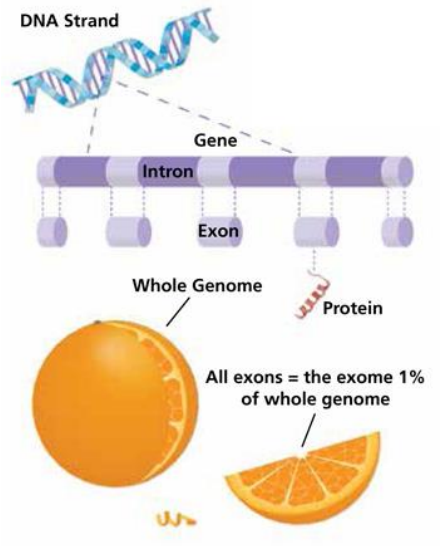
Progressed to chronic encephalopathy with intractable seizures

Parents opted for DNR

Patient passed away at 6 months from respiratory failure with febrile illness

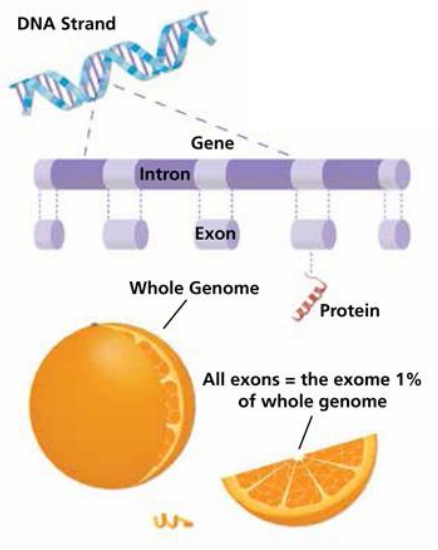
Patient 1 diagnosis

Whole Exome Sequencing (WES)



Patient 1 diagnosis

Whole Exome Sequencing (WES)

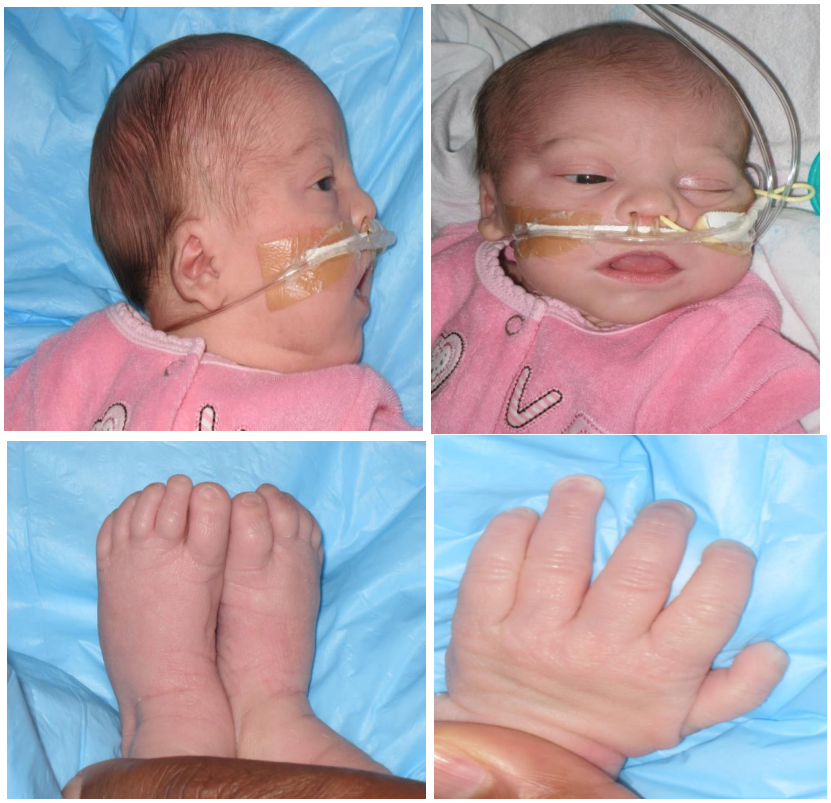


Compound heterozygous mutations in *PIGN*

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

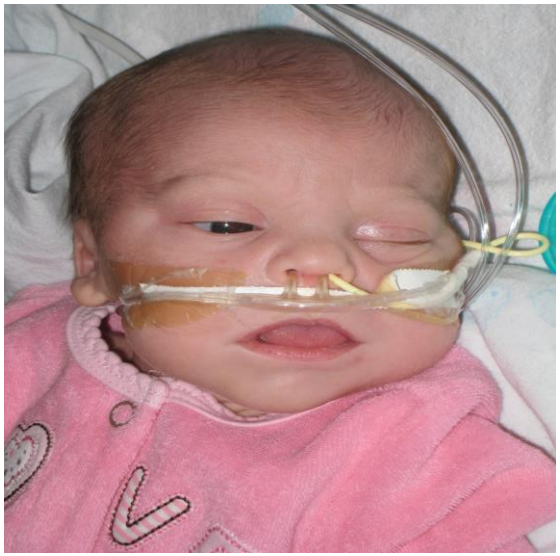
c.2679 C>G (p.S893R)

Patient 2



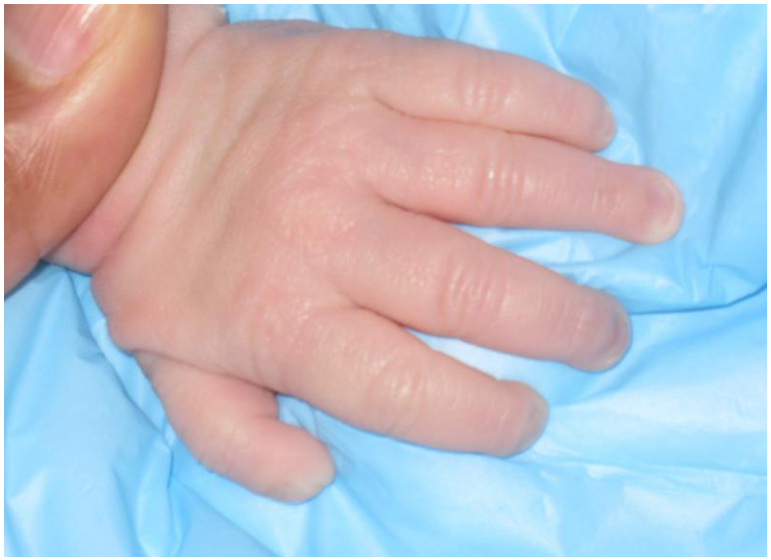
- Hypotonia
- Seizures
- Multiple congenital anomalies

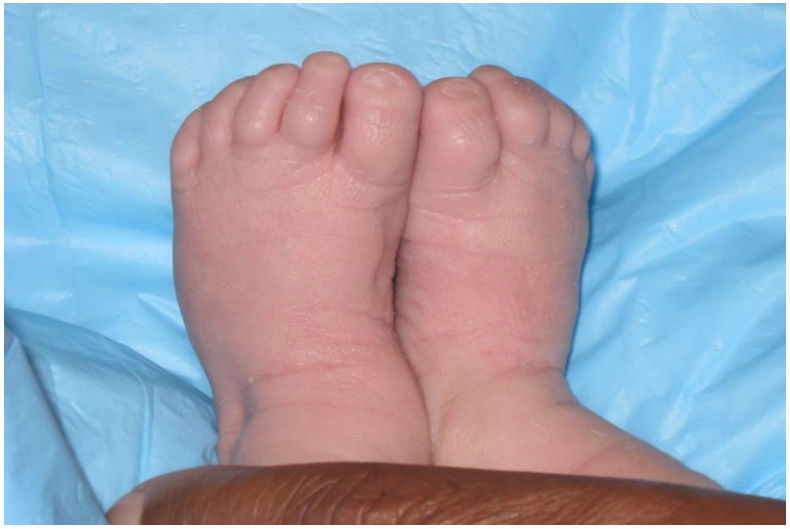
Patient 2



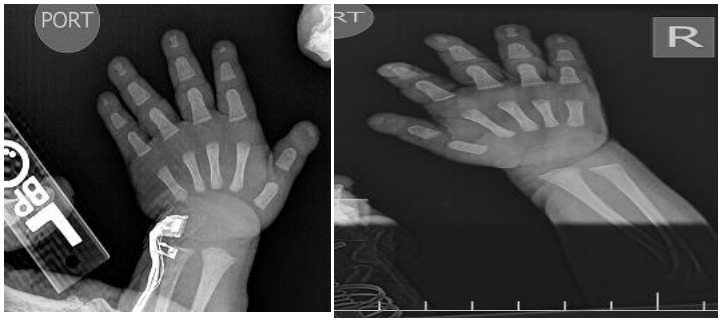
- Large anterior fontanel bitemporal narrowing
- Wrinkled skin around the eyes
- Ears with thickened, over folded helix
- Tented lip

Patient 2





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



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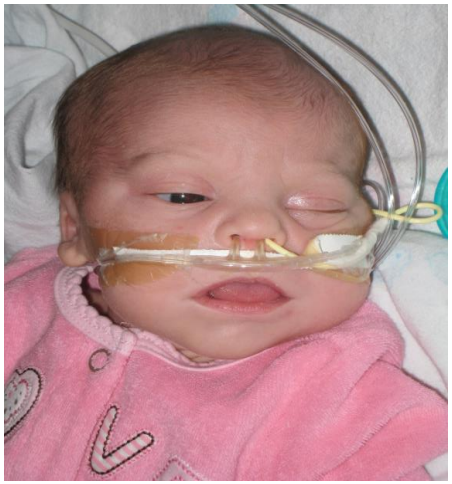
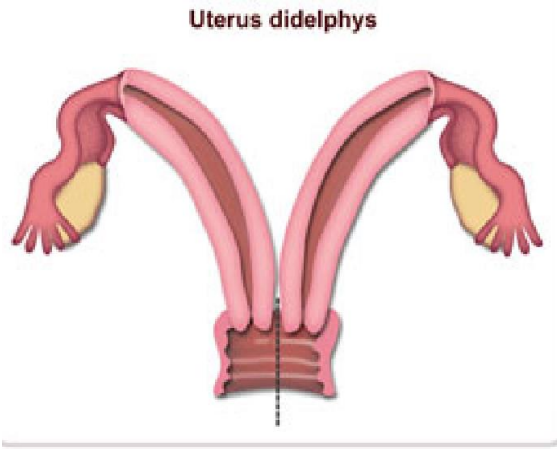
Patient 2





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

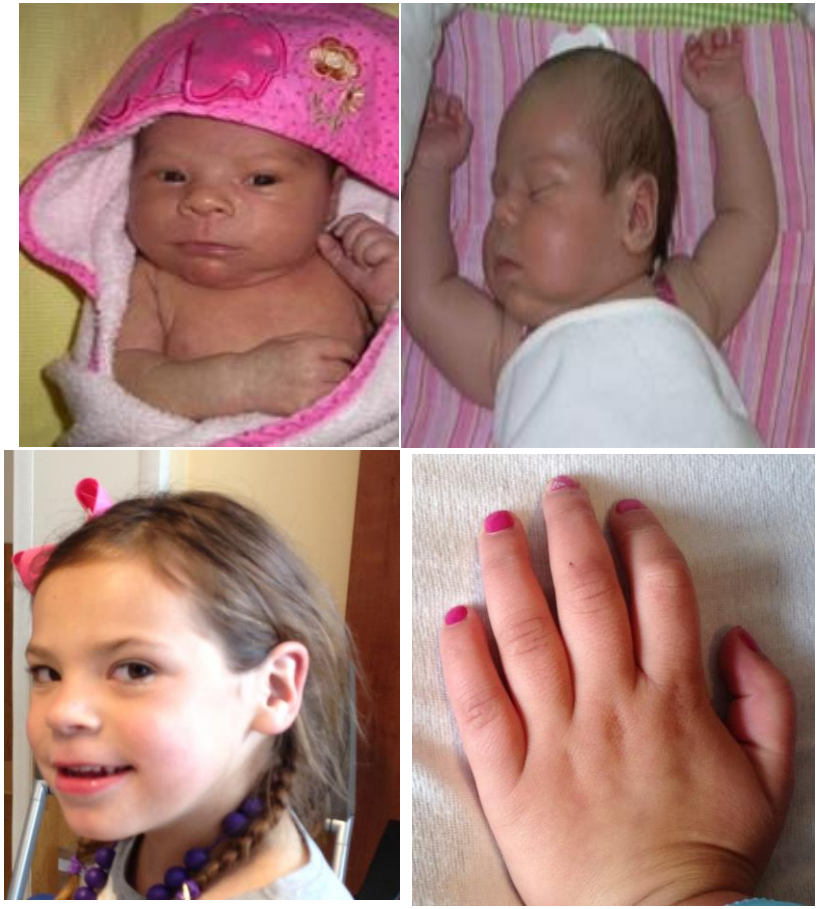
Patient 2 Diagnosis

Compound heterozygous mutations in *PIGN*

c.2126G->A
(p.Arg709Gln)

c.287C>G
(p.Pro96Arg)

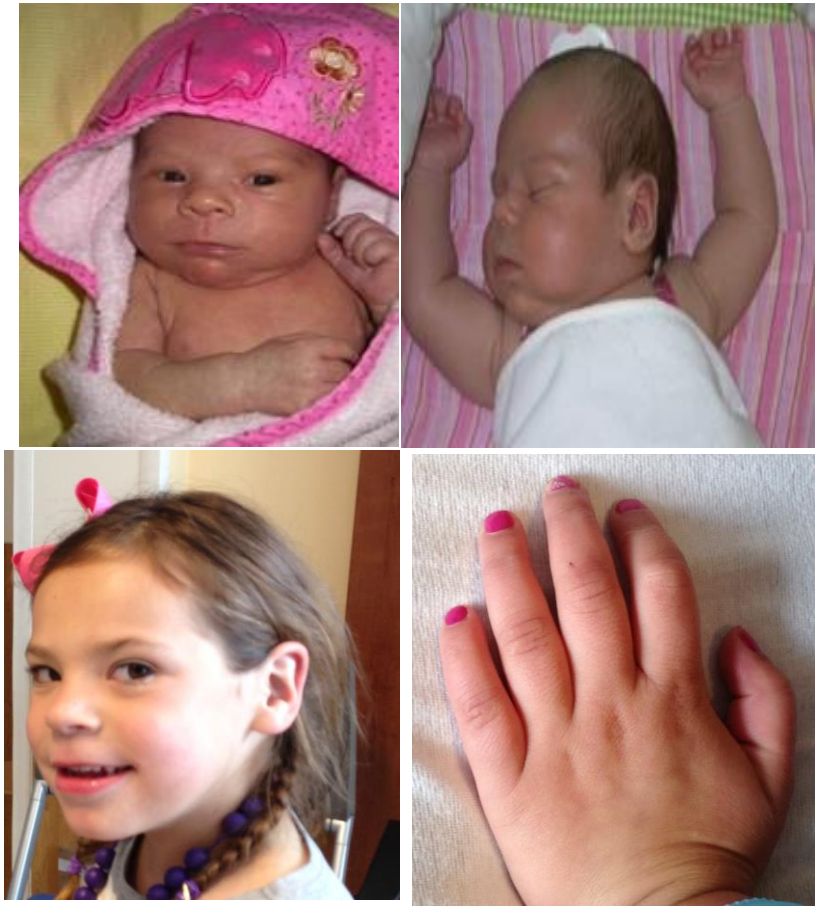
Patient 3



Milder phenotype

- Early onset refractory epilepsy
- Hypotonia
- Developmental delay
- Minor dysmorphic features

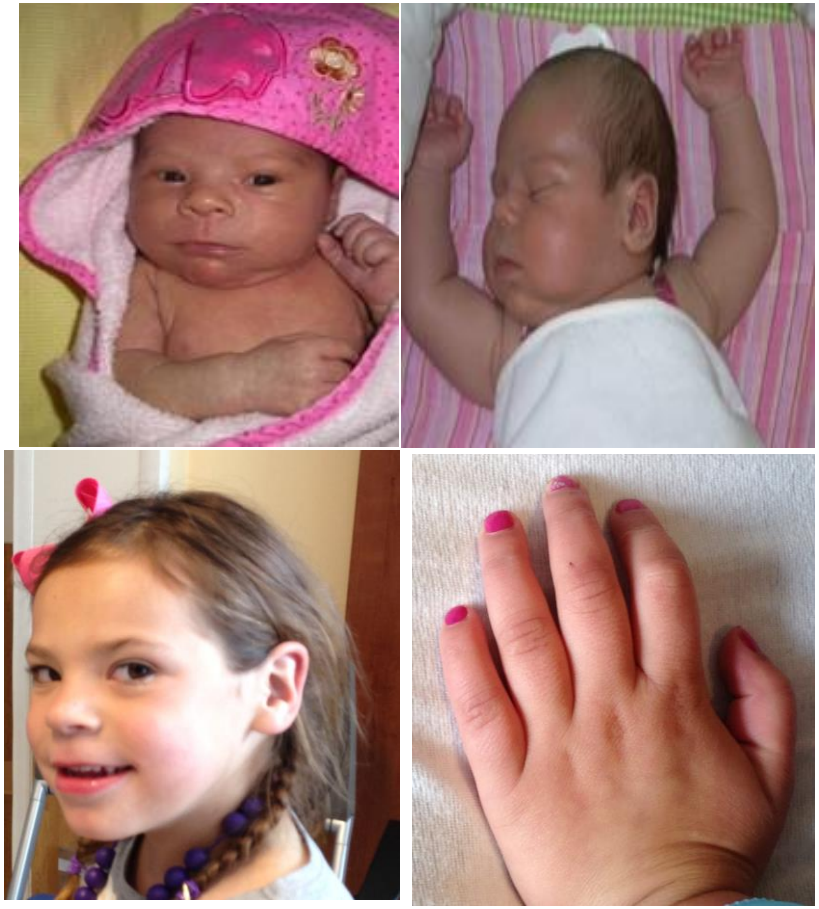
Patient 3



Dysmorphic features:

- Upslanting parbebral fissures
- Long philtrum
- Thin upper lip
- Broad nasal tip and
- 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

GPI biogenesis Defects

- 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

PNH

The Pathobiology of PNH

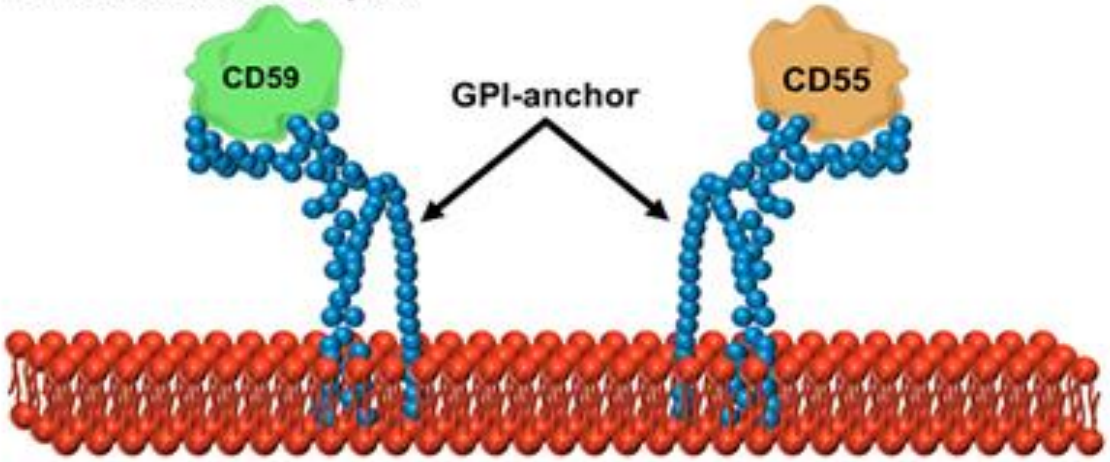
- PIG-A gene is one of >20 genes involved in GPI formation.
- A somatic mutation in the PIG-A gene prevents all GPI-anchored proteins from binding to cell surface

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



Johnson RJ et al. *Mol Pathol*. 2002;55:145-152.[1]
Adapted from Brodsky R. *Paroxysmal Nocturnal Hemoglobinuria*. 2005;419-427.[2]

GPI biogenesis Defects

One limitation in the identification of cases related to GPI deficiencies is the lack of facile and clear biomarkers

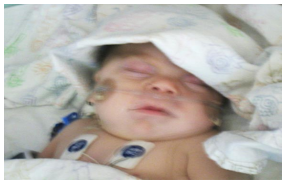
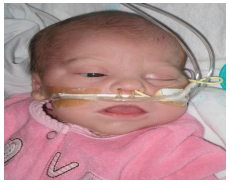
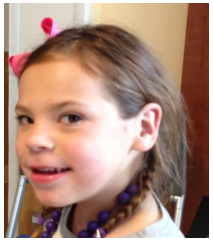
Nearly all the deficiencies have been identified or solved by NGS and not by screening biomarkers

FACS used as functional analysis

- *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

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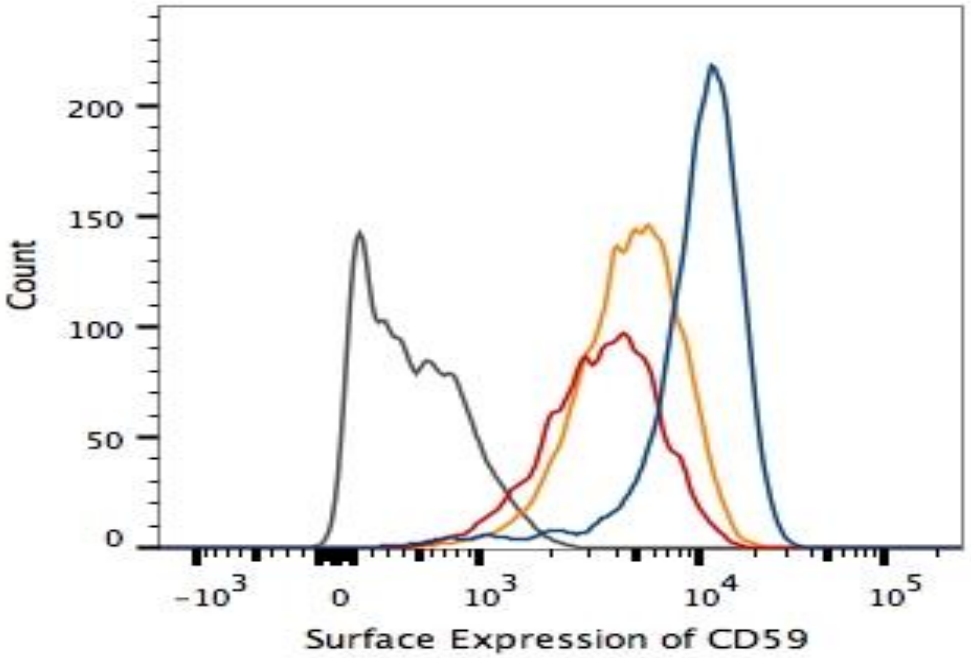
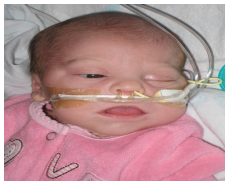
GPI anchored surface markers

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

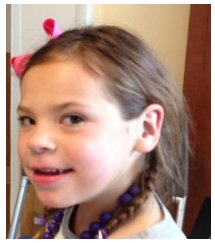
FACS analysis



Patient 2



Patient 3



Normal human fibroblasts

CD59 negative (unstained)

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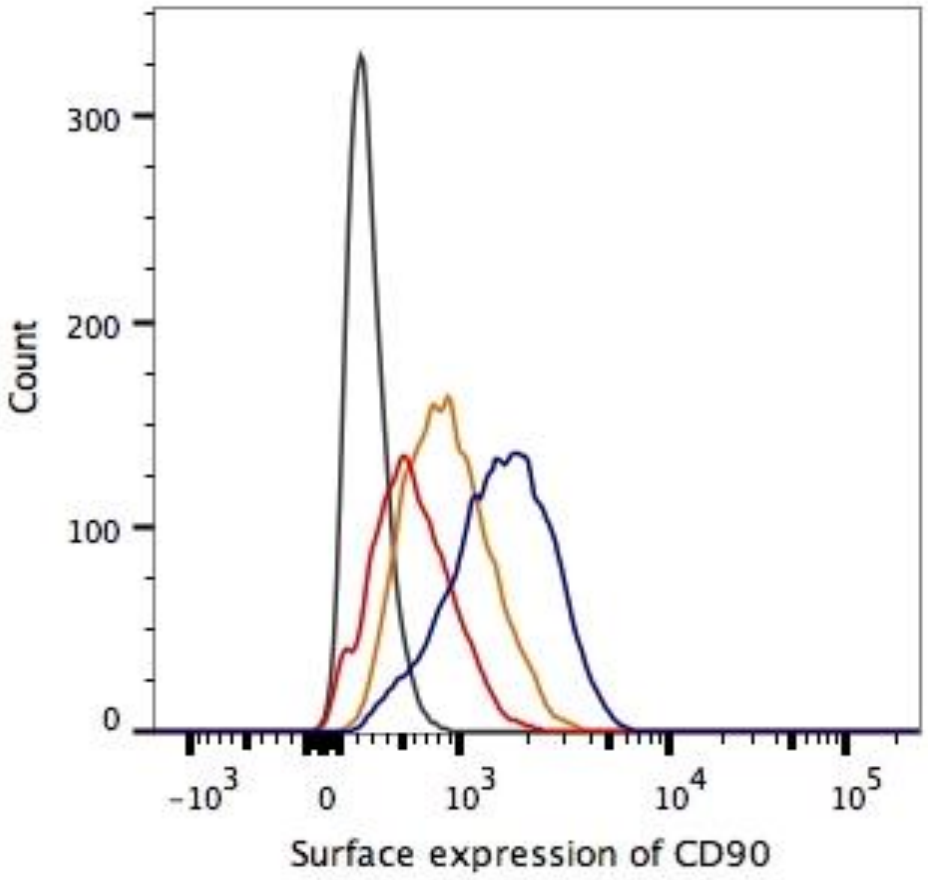
FACS analysis



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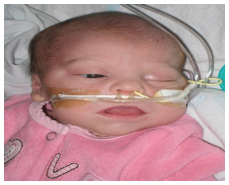

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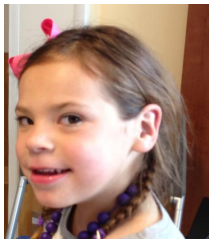


Normal human fibroblasts

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

He Lab CHOP

Xueli Li MD PhD

Rayhan Mohd

Yolanda Foster PhD

Jeshira Reynoso MD

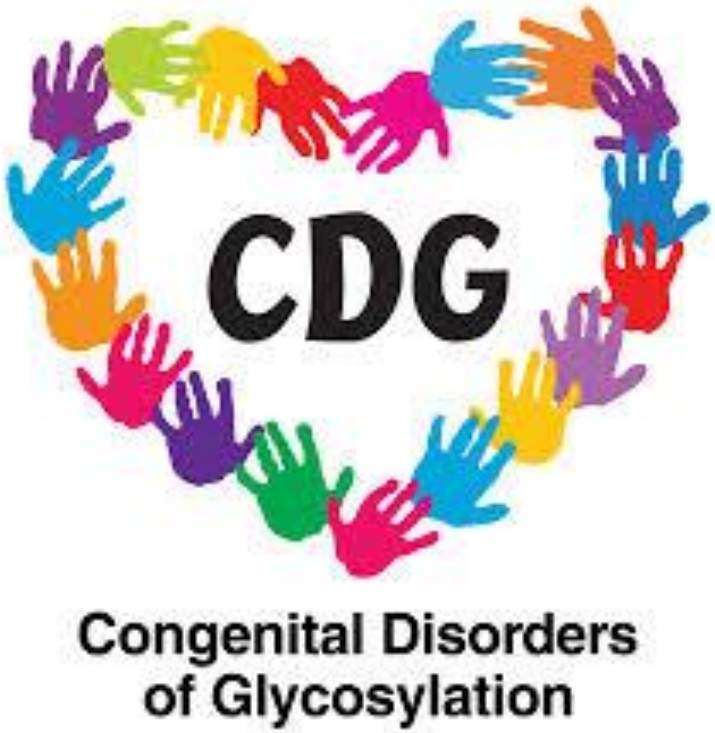
Miao He PhD

Awo Akosua Kesewa Layman MSTP student

Oliver Lab CHOP

Children's Hospital of Philadelphia

Thank you!



Your questions, comments and Ideas are appreciated

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