Congenital disorders of Glycosylation (CDGs): Diagnostic dilemmas

Marli Dercksen (PhD)
28 May 2016
# Points of discussion

- **Introduction**
  - Definitions and facts
  - Biochemical background

- **CDGs**
  - Types
  - Phenotypes and routine biochemical findings
  - Newsflash on novel disorders

- **Diagnostic protocol**

- **CDG landscape in SA**

- **“Treatment” options**

- **Genetic counseling**

- **Prospective prenatal analysis**
Definitions and facts

- **Glycosylation** – Enzymatic post translation modification e.g. proteins, lipids
- 2% of the genome encodes for glycosylation reactions
- 50% of the proteome is glycosylated – Essential for function
- All cells – Cytosol → ER → Golgi apparatus

**Congenital disorder of glycosylation**
- Defective enzymes/transporters in various glycosylation pathways resulting in
- Faulty biosynthesis of glycoproteins (structural and functional) in different compartments of the cell
8 known pathways have been described

Rymen, 2014, Congenital disorder of glycosylation: The Next generation
Congenital disorders of glycosylation (CDGs)

- Table provided summarize current CDG disorders (Approximately 100 and counting)
- New disorder discovered every 17 days
- Inheritance: majority AR and some X-linked
- Nomenclature change: CDG 1c → PMM2-CDG
- N-glycosylation disorders
  - multisystemic with predominant neurological involvement
- O-glycosylation disorders is tissues specific
  - O-mannosylation – Muscular dystrophies
  - Glycosaminoglycan synthesis - bone, cartilage and connective tissue aberrations
  - Glycosphingolipids synthesis – neurological and skin manifestations
- Screening: IEF transferrin serves as gold standard
Biochemical diagnosis

Commercial use of NGS

Identification of glycosylation related disorders (Freeze et al. 2014)

The American Journal of Human Genetics 94, 161–175, February 6, 2014
Phenotype: N-glycosylation disorder

Tell tail sign: Small Cerebellum (hypoplasia/atrophy)

• Primary
  – Microcephaly with/without frontal bossing
  – Psychomotor retardation
  – Facial dysmorphia
    • ears (low set/ epicanthic folds)
    • broad nasal bridge
  – Ophthalmological issues
    • Strabismus
    • RP
  – Seizures – +/- EEG
  – Hypotonia
  – Ataxia
  – Speech delay

• Secondary
  – Body dysmorphism
    • abnormal fat distribution
    • inverted nipples
    • limb abnormalities
  – Stroke-like episodes:
    • mimicking mitochondrial disorders (Briones et al 2001)
  – Peripheral neuropathy
  – Cerebral atrophy
  – Recurrent infection
  – Gastrointestinal symptoms
    • PLE
  – Feeding problems
When to investigate CDG type 2: Can be anything!!!

- Broad spectrum of symptoms including above mentioned
  - not discussed in this lecture
  - not yet identified in SA
- Skin abnormalities
- (Congenital) muscular dystrophy
- Limb-girdle dystrophy
- Skeletal dysplasia syndromes e.g. cutis laxa
- Ehlers-Danlos syndrome
Routine biochemistry: Helpful hints

CDG type 1
- Hypoalbuminemia
- ↓ Coagulation factors: protein S & C, factor XI
- Hypoproteinemia (↓ ATIII)
- Abnormal liver enzymes
- Endocrinological abnormalities
  - Thyroid Hormones
  - Reproductive hormones
  - Cortisol etc.
- Hypocholesterolemia
- + CDT test
- Recently: + IRT (NBS)
- Mostly + IEF transferrin (May be normal in some cases)

CDG type 2
- ↓/↑ Coagulation factors – less than type
- Abnormal liver enzymes
- Unspecific endocrinological abnormalities
- Unidentified blood group
- Leukocytosis
- Differential anaemia
- Thrombocytopenia
- Vit K deficiency
- +/- CDT test
- +/- IEF transferrin

Always exceptions to the rule!!
Novelties in the field of CDGs

• **Secondary glycosylation disorders** including
  – GALE and GALT deficiency
  – Phosphoglucomutase-1 (PGM1) deficiency – Previously recognized as GSD XIV. **Clinical note:** Cleft palate and myopathy
  – Phosphoglucomutase-3 (PGM3) deficiency – **Clinical note:**↑ IgE

• Certain syndromes associated with defective glycosylation
  – **Congenial myasthenic syndrome** (CMS) associated with DPAGT1-CDG
  – More may be discovered……..

• **Novel De-glycosylation disorder** due to glycanase deficiency (Mutations in NGLY1 gene)
  • Similar symptomatic presentation as CDG type 1.
  • Liver storage disease - Accumulation of abnormal glycoproteins in cytosol of cell due to defective degradation of misfolded glycoproteins
  • [http://www.ngly1.org](http://www.ngly1.org) – looking for more patients
Clinical suspicion of CDG

IEF serum transferrin on 2 independent samples

Type 1 pattern
- ALG6 gene sequencing (SA patients)
- PMM2 gene (due to high prevalence (1:20 000))
  Negative

Type 2 pattern

Normal
- Clinical suspicion +++
- Other possible IEMs was excluded

Gene panel testing

Candidate genes?
- Yes
- No

WES/WGS?
- Yes
- No

Variant already described
- Yes
- No

Report
- Yes
- No

Research/Recommendations

Adapted figure: Rymen, 2014, Congenital disorder of glycosylation: The Next generation
CDG landscape in South Africa

1998-2001: First South African CDG case described in SA

CDG screening method was validated on DBS

Speculation on type – 1a/1b?? – Genotype remained unknown.

“……This open a whole new chapter in the study of genetics and metabolic disease in this country……”

After publication of paper – ALG6 gene was sequenced and mutations were identified confirming CDG 1c.
CDG landscape in South Africa

• 2001-2007: Biochemical level identification. No Genotyping done
• 2008-2013 – more in depth studies into the genotype-phenotype of SA CDG patients


ALG6-CDG in South Africa: Genotype-Phenotype Description of Five Novel Patients.

Derksen M¹, Crutchley AC, Honey EM, Lippert MM, Matthijs G, Mienie LJ, Schuman HC, Vorster BC, Jaeken J.

- ALG6-CDG Genotype-Phenotypes in Caucasian population were identified
  • Severe neurological-gastrointestinal phenotype (unique in SA)
  • Classical neurological type seen in European countries
- Founder effect was suggested
- Most common CDG type in SA. Definitely the most diagnosed CDG type in SA and second most in the world (prevalence <1/1 000 000).
<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender and age</strong></td>
<td>Male, died at 5 months</td>
<td>Male, died at 3.1 years</td>
<td>Male, 3.1 years</td>
<td>Female, 6.9 years</td>
<td>Male, 3.9 years</td>
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<tr>
<td><strong>Neurological</strong></td>
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<td><strong>abnormalities</strong></td>
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<tr>
<td>Microcephaly</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hypotonia</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Strabismus (internal or external)</td>
<td>–</td>
<td>+ (internal)</td>
<td>–</td>
<td>+ (internal)</td>
<td>+ (internal)</td>
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<tr>
<td>Psychomotor retardation</td>
<td>(+++</td>
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<td>(including speech delay)</td>
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<tr>
<td>Epilepsy</td>
<td>–</td>
<td>+++</td>
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<tr>
<td>Hypokinesia/Tremors</td>
<td>–</td>
<td>–</td>
<td>Hypokinesia</td>
<td>Hypokinesia</td>
<td>Hypokinesia</td>
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<tr>
<td>EEG</td>
<td>Not performed</td>
<td>Abnormal</td>
<td>Abnormal</td>
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<td>Abnormal</td>
</tr>
<tr>
<td>Brain imaging</td>
<td>Thin corpus callosum</td>
<td>Mild cerebral atrophy</td>
<td>Mild cerebral atrophy</td>
<td>Mild cerebral atrophy</td>
<td>Severe atrophy, small cerebellum</td>
</tr>
<tr>
<td>Optic dysfunction/atrophy</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Dysmorphisms</td>
<td>Enlarged eyes, micrognathia, broad-nasal bridge; camptodactyly (observed in fingers)</td>
<td>Broad nasal bridge</td>
<td>Broad nasal bridge; inverted nipples</td>
<td>Broad nasal bridge; small feet and fat pads on finger tips</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>Protein-losing enteropathy with severe edema and ascites</td>
<td>–</td>
<td>Neonatal constipation and hepatomegaly</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Other symptoms</td>
<td>Poor feeding</td>
<td>Recurrent infections</td>
<td>Poor feeding; recurrent infections</td>
<td>Recurrent infections</td>
<td>Poor feeding; recurrent infections</td>
</tr>
</tbody>
</table>

Patients 1 to 5 represent CDG cases in five separate families.
Current CDG status in SA

- Current difficulties in diagnosis
  - False positives: Patient with secondary liver involvement, alcohol abuse (FAS???) and galactosemia patients on galactose-free diet
  - Limitations in CDG screening in SA
  - Overlap in clinical presentation

- Current amount of patients fitting CDG criteria
  - Various patients (>763) with CDG phenotype has been screened
  - Abnormal CDG screening, no genotype yet = 30+ patients (all population groups)
  - Confirmed ALG6 (CDG 1c) cases = 12 (About 1-2 cases per year)
  - Confirmed PMM2 (CDG 1a) cases = 1 (Caucasian)

- Continuous CDG collaboration with Belgium group: genotyping, prenatal analysis and research

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**Epilepsy, proximal muscle weakness and ataxia in ALG6-CDG; recognizable phenotype and unique malformations**

Eva Morava 1,24*, Vera Tiemes1,2, Christian Tiel9, Natalie Seta4, Pascal de Lonlay5, Hans de Klerk6, Margot Mulder7, Estella Rubio8, Gepke Visser9, Peter van Hasselt9, Dafne DG Horovitz10, Carolina Fischinger Moura de Souza10, Ida VD Schwartz10, Andrew Green11, Mohammed Al-Owain12, Gracielle Uziel13, Sabine Sigaudi14, Brigitte Chabrol15, Franc-Jan van Sпрonserн16, Martin Steinert17, Eleni Kominis18, Donald Wurm19, Andrea Bevo120, Jaak Jaekens21, Marli Derksen21, Karen Huijben2, Gert Matthijs22, Dirk J Lefebre23, Ron A Wevers2*

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“Treatment” options

Treatable CDGs

- PMI deficiency (CDG 1b) with mannose
- GDP-fucose transporter deficiency with fucose
- phosphoglucomutase-1 (PMG1) deficiency with galactose

Secondary therapeutic management

- **Dietary tolerance**: Carbohydrates, proteins and fats. Tube feeding may be required (Ketogenic diet is contra-indicated)
- **“Infantile catastrophic phase”**: Infection, seizures and hypoalbuminemia. Hospitalize immediately. Some patients respond to albumin replacement
- **Occupational and physiotherapy therapy**
- **Ophthalmological assistance**: Glasses, patching or surgery may be option
- **Hypothyroidism**: Treatment reserved for patients with elevated TSH and low free thyroxin
- **Coagulopathy**: Surgery complications – consult hematologist. Look out for signs of deep vein thrombosis
- **Epilepsy**: Anti-convulsants may help some patients, but most are resistant
- **Gastro-intestinal problems**: Mannose/Octreotide may help for PLE
Genetic counseling and prenatal testing

- **Genetic counseling** is necessary due to detrimental outcome

- **Prenatal testing**
  - Since 2011: It is possible if mutations of index patient is genetically confirmed (parents should also be typed)
  - Chorion villi sample is needed (13 weeks) – high risk procedure
  - If this procedure fails – amniocytes may also be used
  - Again emphasis on counseling – very traumatic for the parents
  - Preimplantation screening is now an option??!!
World Congenital Disorders of Glycosylation (CDG) Awareness Day

CDG families, friends and professionals around the world will participate in celebrating the World Congenital Disorders of Glycosylation (CDG) Awareness Day on May 16. This action aims at reinforcing the request directed to have this day legally recognized by the World Health Organization (WHO).

http://comunicarciencia.bsm.upf.edu/?p=5181
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- All CDG families
- Doctors and genetic counselors (special thanks to Dr. Lindsay Lambie and Dr. Engela Honey) for their expert advise to the CDG families

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Thank You