

# Diagnosis of CDG Enzyme Analysis and Other Investigations

**Biochemical Genetics Network**

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# EUROGLYCANET

- European Consortium of labs working on CDG (about 29 participants) (Research & Diagnosis)
- London – Core Lab (with 5 others in Europe)
- Professor Bryan Winchester – UK Coordinator
- ‘Diagnostic Carousel’
- International Patient Database
- Website [www.euroglycanet.org](http://www.euroglycanet.org)

# Abnormal Transferrin Glycoform Pattern Type I

Next step towards diagnosis?

# Enzymology to exclude CDG-Ia & CDG-Ib

- Notify **Prof. Bryan Winchester** (ICH)
- **Contact Enzyme Laboratory (GOSH/ICH)**
- **Marie Jackson** (Clinical Scientist)
  - Sample requirements for enzymology
    - Leucocytes (5-10 ml blood in Li Heparin)
- **Derek Burke** (BMS 3)
  - If fibroblast culture is required

# PMM or PMI deficiency

- Phosphomannomutase deficiency
  - Diagnosis of CDG-Ia
- Phosphomannose isomerase deficiency
  - Diagnosis of CDG-Ib (Treated by mannose therapy)
- Diagnosis must be confirmed by mutation analysis if PND is to be offered in the future

# CDG-Ia/Ib - further investigations

- Mutation analysis (Leuven, Belgium via ICH)
  - DNA or 5ml blood in EDTA for DNA extraction
  - **PMM2** gene (CDG-Ia)
  - **PMI1 (MPI)** gene (CDG-Ib)
- Samples from both parents are required before PND can be offered
  - 10ml Li Heparin (Enzymology)
  - 5ml EDTA (DNA analysis)
- **NOTE:** investigations should be completed before the mother becomes pregnant again

**PMM and PMI normal**

**Next step - LLO analysis**

**LLO = Lipid-Linked Oligosaccharides**

# Glycosylation Pathway

## Stage 1

- Activation of sugar precursors
- Assembly of oligosaccharides on ER membrane (lipid-linked) Lipid = dolichol
- Transfer to the nascent polypeptide

## Stage 2

- Processing of N-linked glycans in the Golgi

# Lipid-Linked Oligosaccharides

- **LLOs are intermediates in the first stage of the glycosylation pathway**
- **Specific LLOs will accumulate if there is a block in the pathway due to an enzyme defect**

# LLO Analysis

**Zurich, Switzerland via ICH, London**

- Patient's skin fibroblasts growing in culture
- Labelled with [ $^3\text{H}$ ]-mannose
- LLOs extracted
- HPLC analysis
- “A specifically altered LLO profile can be diagnostic for a given type of CDG”

# Yeast Glycosylation Mutants

- LLO profile from a yeast mutant will reflect the specific enzyme defect
- Similar LLO profile in fibroblasts from a patient with the same defect

E.g. CDG-Ii fibs and yeast *alg2* mutant have the same LLO profile and a deficiency in the same mannosyl transferase.

# Gene Analysis & Complementation Studies

- LLO profile – predicts defective glycosyl transferase
- Mutation analysis of relevant gene
- Yeast mutant (temp. sensitive) studies E.g.
  - alg2* yeast + wild type h*ALG2* gene (**temp sens corrected**)
  - alg2* yeast + defective h*ALG2* gene (**remains temp sens**)
- Confirms that the mutant gene is the cause of the glycosylation defect

**Abnormal Transferrin  
Glycoform Pattern  
Type II**

**Next step – Glycan Analysis**

# Glycosylation Pathway

## Stage 1

- Activation of sugar precursors
- Assembly of oligosaccharides on ER membrane (lipid linked).
- Transfer to the nascent polypeptide

## Stage 2

- Processing of N-linked glycans in the Golgi (addition and removal of sugars to form mature branched glycans, involving glycosyl transferases and glycosidases)

# N-linked Glycans

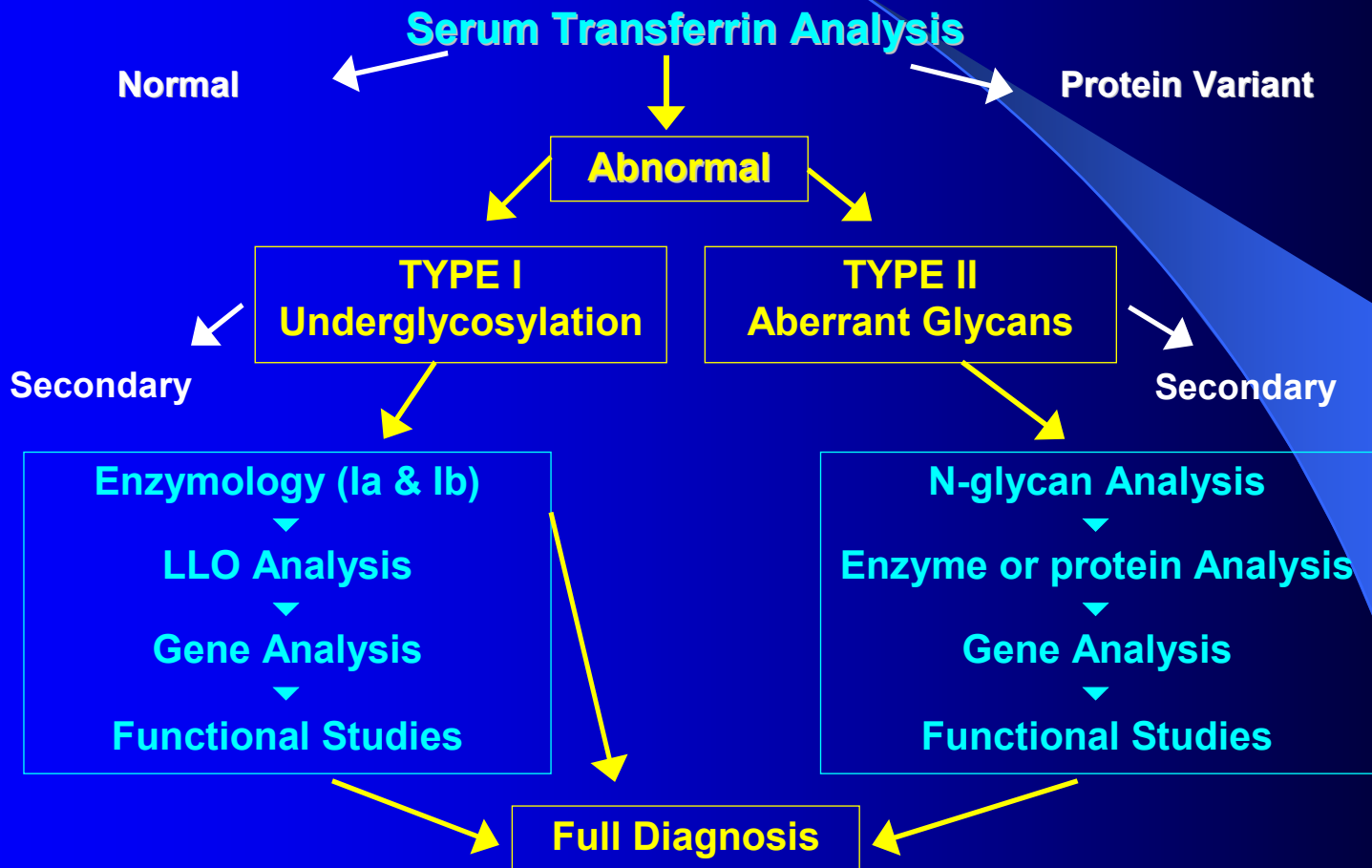
- N-linked glycans are ‘intermediates’ (attached to the growing polypeptide chain) in the second stage of the glycosylation pathway
- Defects in enzymes involved in glycan processing or defects in Golgi integrity may cause accumulation of aberrant N-linked glycans

# N-linked Glycan Analysis

## Philippa Mills ICH, London

- Plasma from affected patient
- Enzymatic release of glycans from proteins
- Purified glycans analysed by MALDI-TOF-MS
- Glycans structures characterised
- Enzymology/further studies  Europe

# CDG Screening & Diagnosis



# UK Contacts - London

## EUROGLYCANET Co-ordinator for the UK

Bryan Winchester<sup>3</sup>

### Clinical Advice

- Stephanie Grunewald<sup>1</sup>
- Peter Clayton<sup>1</sup>

### Transferrin Analysis

- Geoff Keir<sup>2</sup>
- Viki Worthington<sup>2</sup>

### Enzymology (Ia & Ib)

- Marie Jackson<sup>1</sup>

### Fibroblast culture

- Derek Burke<sup>1</sup>

### Glycan Analysis

- Philippa Mills<sup>3</sup>

1. GOSH/ICH

2. NHNN

3. ICH