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 $[^3\text{H}]-\text{mannose}$
- 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. 
  \textit{alg2} yeast + wild type h\textit{ALG2} gene (temp sens corrected)
  \textit{alg2} yeast + defective h\textit{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

Serum Transferrin Analysis

Normal

Abnormal

Type I
Underglycosylation

Enzymology (Ia & Ib)
- LLO Analysis
- Gene Analysis
- Functional Studies

Full Diagnosis

Type II
Aberrant Glycans

N-glycan Analysis
- Enzyme or protein Analysis
- Gene Analysis
- Functional Studies

Protein Variant

Secondary

Secondary
UK Contacts - London

EUROGLYCANET Co-ordinator for the UK
Bryan Winchester

Clinical Advice
- Stephanie Grunewald
- Peter Clayton

Transferrin Analysis
- Geoff Keir
- Viki Worthington

Enzymology (Ia & Ib)
- Marie Jackson

Fibroblast culture
- Derek Burke

Glycan Analysis
- Philippa Mills

1. GOSH/ICH
2. NHNN
3. ICH