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CDG – HPLC

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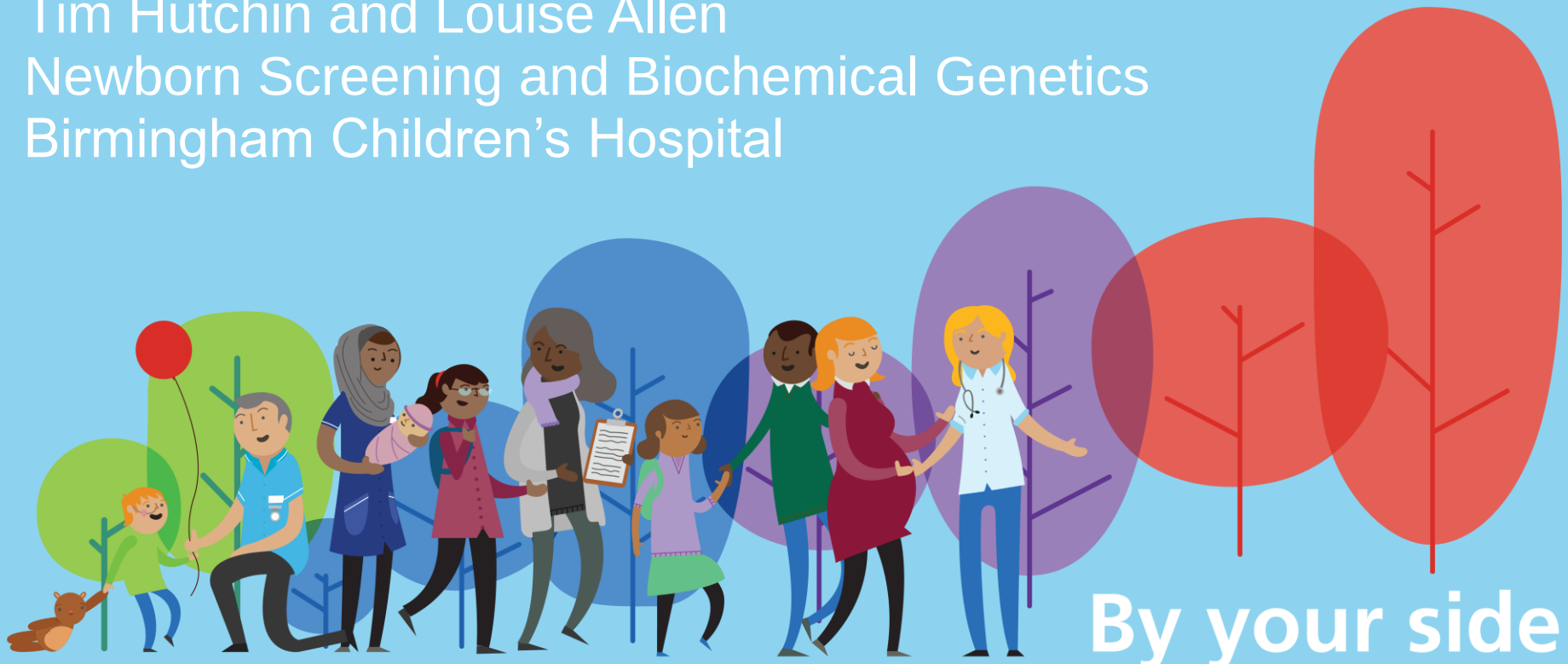

Case presentation

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6yr old female

Global developmental delay
Late walker
Speech delay
Ataxia- poor balance and intermittent tremor particularly her hands
Generally clumsy
Factor XI deficiency

Squint which was operated on, wears glasses now

MRI showed cerebellum atrophy bilaterally and symmetrically

Analysis of transferrin isoforms

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	Patient	Normal range
T0	2.29%	0
T1	-	0
T2	10.93%	0.6 - 1.9%
T3	1.83%	1.9 - 7.4%
T4	73.89%	75.2 - 83.7%
T5+6	11.05%	11.2 - 19.4%

Consistent with a Congenital Disorder of Glycosylation (CDG) Type I

Further investigations

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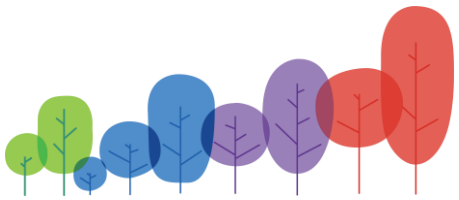
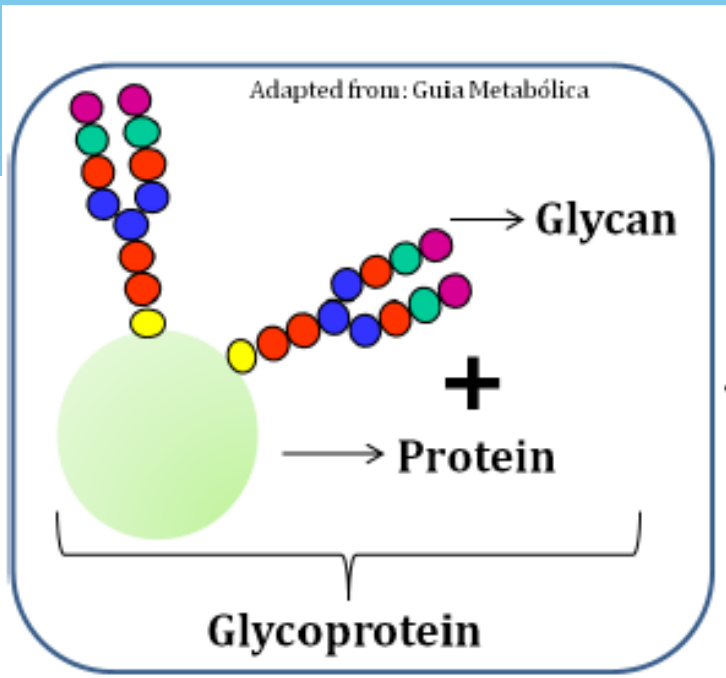
	Patient	Normal
Phosphomannomutase (CDG-1a)	0.3	0.5-5.3 mU/mg protein
Phosphomannose isomerase (CDG-1b)	26.2	10.9-43.7 mU/mg protein
Phosphoglucomutase (CDG-1t)	296	94-424 mU/mg protein

CDG-PMM (1a) confirmed by DNA
heterozygous for c.422G>A (p.Arg141His) and c.722G>C (p.Cys241Ser) in the PMM2 gene



Glycosylation



Glycosylation is an important form of post translational modification by which a carbohydrate(glycan) is covalently attached to a target macromolecule, typically proteins and lipids.

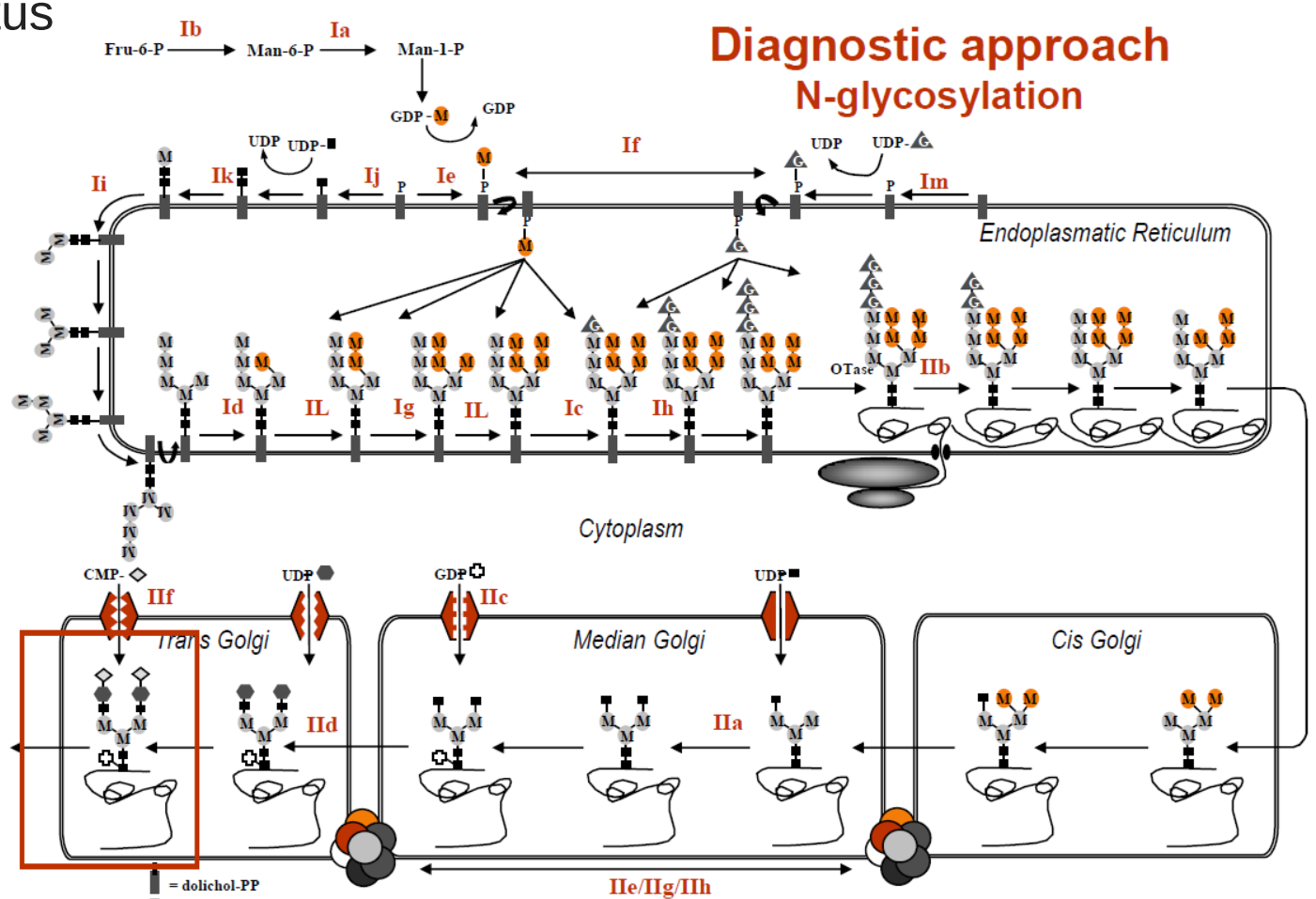
The resulting glycoproteins and glycolipids have numerous important functions in all tissues and organs, e.g.

- Important for correct folding and stability of proteins
- Cell-to-cell adhesion- used by cells of the immune system
- Glycosylation underpins the ABO blood group system
- Transport (eg transferrin)
- Hormones
- Regulation of development

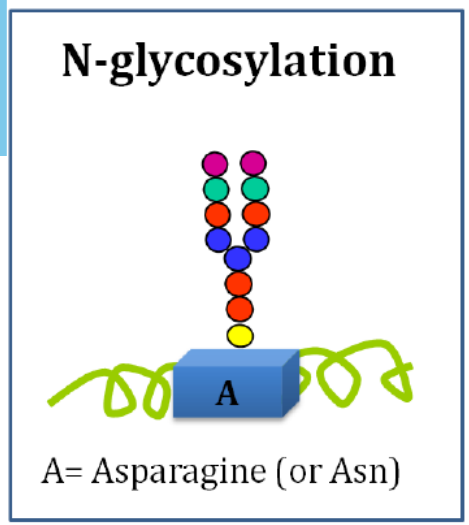
Glycosylation is a complex process

200-400 genes involved (1-2% of all human genes).

Glycosylation of proteins and lipids occurs in the endoplasmic reticulum and golgi apparatus



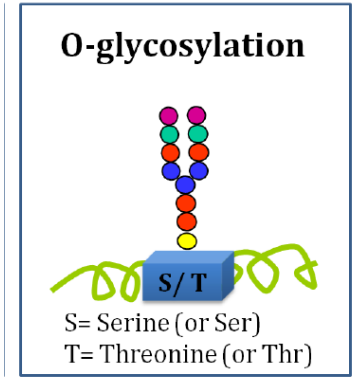
Over 170 different CDGs have been described



Disorders of protein N-glycosylation

About half of CDG are defects of N-glycosylation.
Incidence may be as high as 1:22,000
Further divided into two subtypes:

- type 1 - defects of oligosaccharide assembly and transfer (ER defects)
- type 2 - defects in oligosaccharide processing after they are bound to proteins (golgi defects)



Disorders of protein O-glycosylation

Disorders of glycosphingolipid and GPI-anchor glycosylation

Defects of multiple glycosylation and other pathways

Most CDGs are untreatable

CDG nomenclature

Originally named CDG1a, 1b….
CDG2a, 2b etc

But are now called after the gene

Old name	new name
CDG1a	PMM2-CDG
CDG1b	MPI-CDG
CDG1q	SRD5A3-CDG
CDG2a	MGAT2-CDG
CDG2e	COG7-CDG
CDG2m	SLC35A2-CDG

CDG SHOULD BE CONSIDERED IN EVERY PATIENT WITH AN UNEXPLAINED SYNDROME

- DEVELOPMENTAL DELAY
- HYPOTONIA

- SEIZURES
- STROKE-LIKE EPISODES
- CEREBELLAR HYPOPLASIA
- DYSMETRIA
- ATAXIA
- PERIPHERAL NEUROPATHY

- STRABISMUS
- NYSTAGMUS

- SCOLIOSIS

- PERICARDIAL EFFUSION

- ABNORMAL SUBCUTANEOUS FAT PATTERN
- ABSENCE OF PUBERTAL DEVELOPMENT
- INVERTED NIPPLES

- ABNORMAL HEMATOLOGICAL AND ENDOCRINE PARAMETERS
  - LOW SERUM CONCENTRATION OF FACTORS XI ANTITHROMBIN III, PROTEIN C AND/OR PROTEIN S
  - LOW TOTAL SERUM THYROXINE WITH MOSTLY NORMAL FREE SERUM THYROXINE

- HEPATOMEGALY

- FAILURE TO THRIVE
- PROTEIN LOSING ENTEROPATHY

- ABNORMAL BIOCHEMICAL PARAMETERS
  - INCREASED SERUM TRANSAMINASES
  - LOW SERUM CHOLESTEROL

- OSTEOPENIA

From The Portuguese Association for CDG and other Rare Metabolic Diseases (APCDG-DMR)

Testing for CDGs

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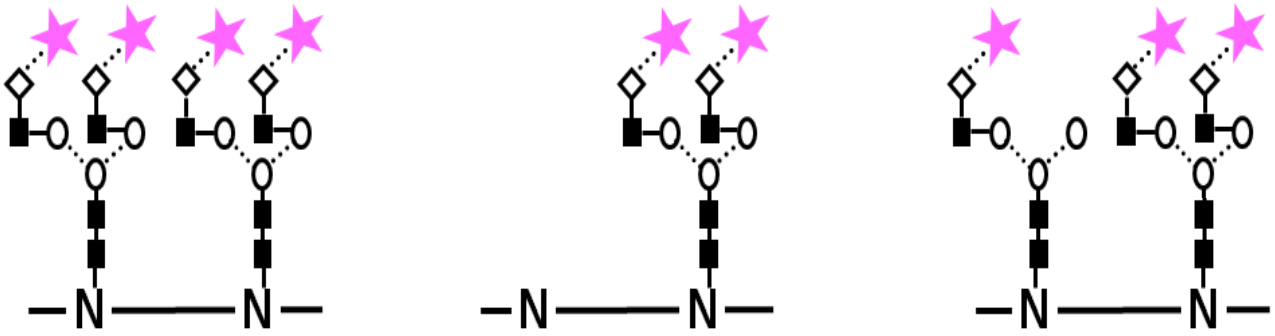
Serum transferrin is a glycosylated protein which can be used as a first line test to detect most N-glycosylation defects

CDG patients may have reduced sialylation of transferrin (and other proteins); either due to hypoglycosylation (type I) or abnormal glycan structure (type II)



Normal **Type 1** **Type 2**

predominant glycoforms

T4 **T2 and T0** **T3 and T1**

Most commonly used methods are isoelectric focusing, CE and HPLC

http://cdgcare.com/what-is-cdg/

HPLC method

- Chromsystems CDT kit (validated)
- Biomarker for Chronic Alcohol Abuse
- Quick and simple prep
- 20 mins running time
- High salt elution – need for water washes
- Issues with ERNDIM EQA (sample size)

CHROMSYSTEMS®
DIAGNOSTICS BY HPLC & LC-MS/MS

Equipment and Kit

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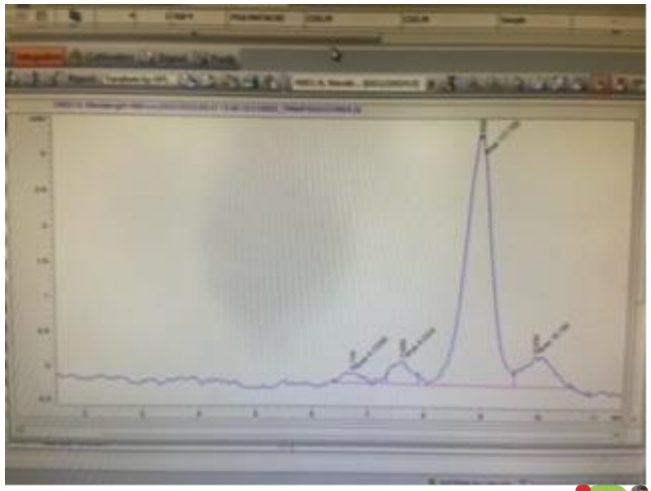

Methodology

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- Prepare reaction mix containing precipitation, stabilisation and neutralisation buffers.
- 50ul RM added to 100ul sample
- Lipoproteins are removed and the transferrins are saturated with iron
- This ensures stable chromatography
- Ternary system Mobile Phase A and B (separation) and Mobile Phase C washes the column
- UV detector wavelength 460nm

Normal Pattern

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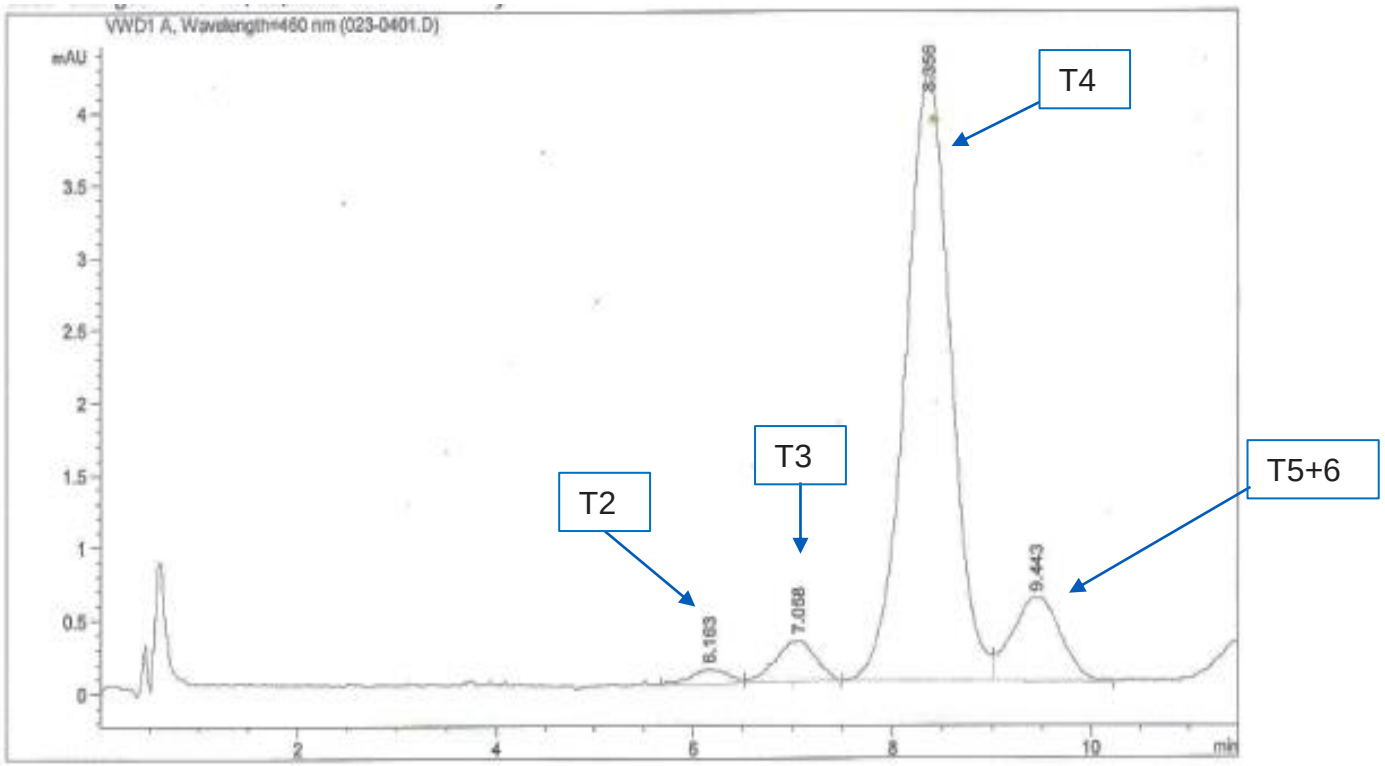
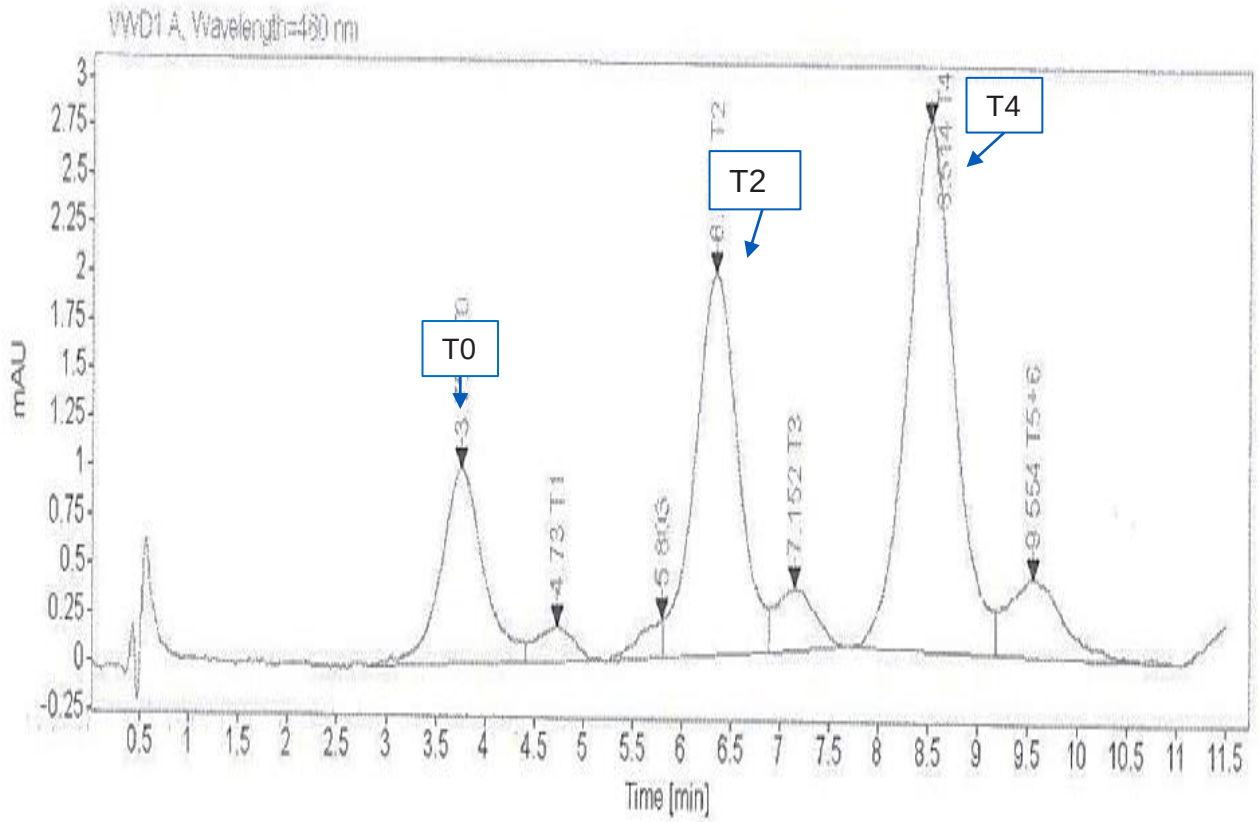


Normal plasma/serum should show the tetra-sialo glycoform (T4) as the predominant peak with significantly smaller peaks for the disialo (T2) tri- sialo (T3) and penta+hexa sialo (T5+6) glycoforms.

CDG TYPE I pattern



A Type I CDG sample will typically show an asialotransferrin peak (T0) with an increased disialotransferrin peak (T2) and a smaller than normal T4 peak

CDG Type II pattern

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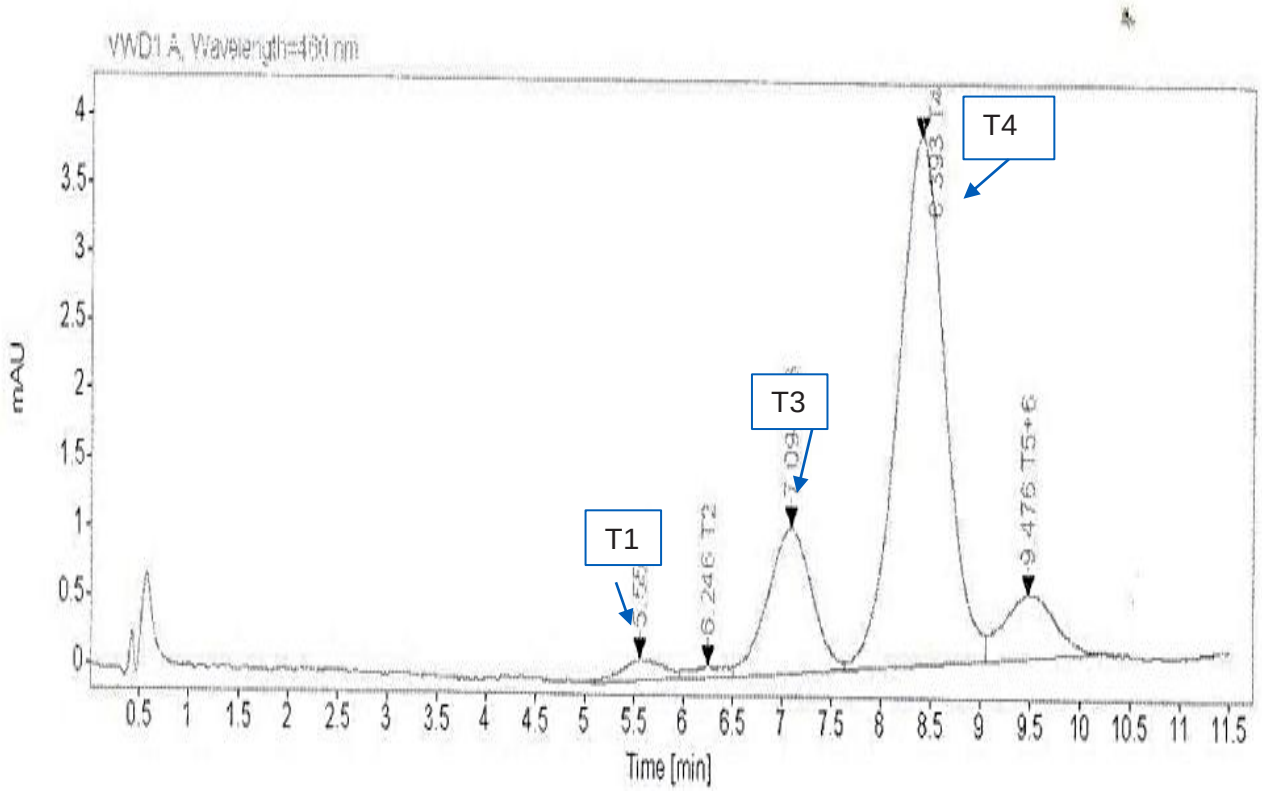
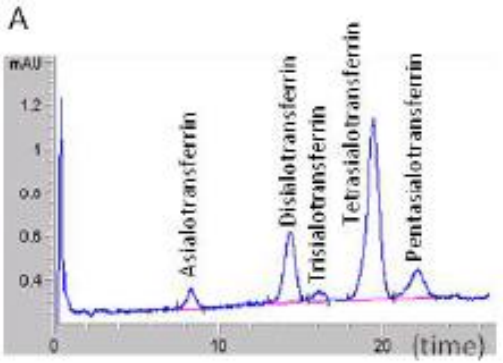
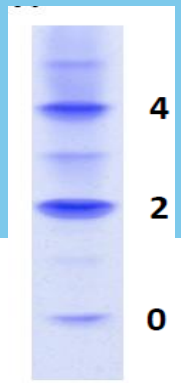
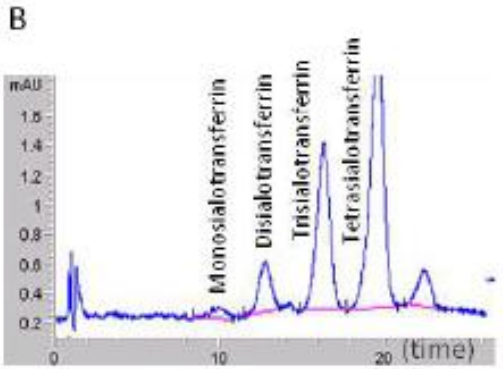


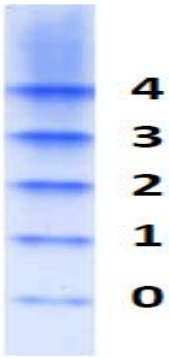
A Type II CDG sample will typically show an increased trisialotransferrin peak (T3), with a smaller than normal T4 peak. Asialo (T0) and mono sialotransferrin (T1) glycoforms may also be present.



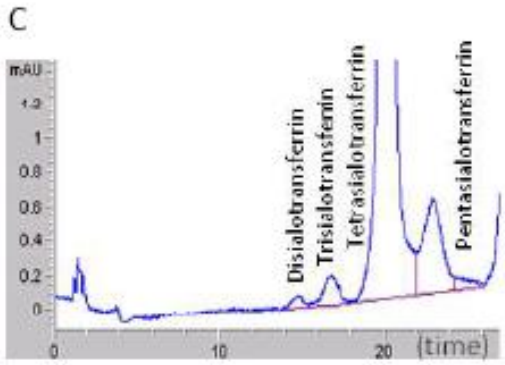


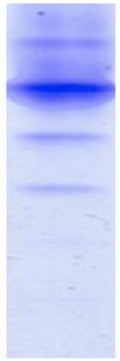
Type 1





Type 2

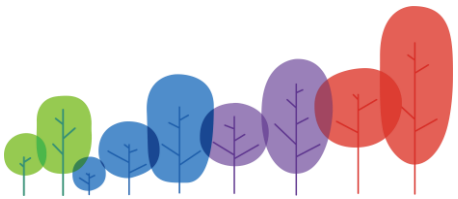




Normal profile

HPLC

IEF



Limitations

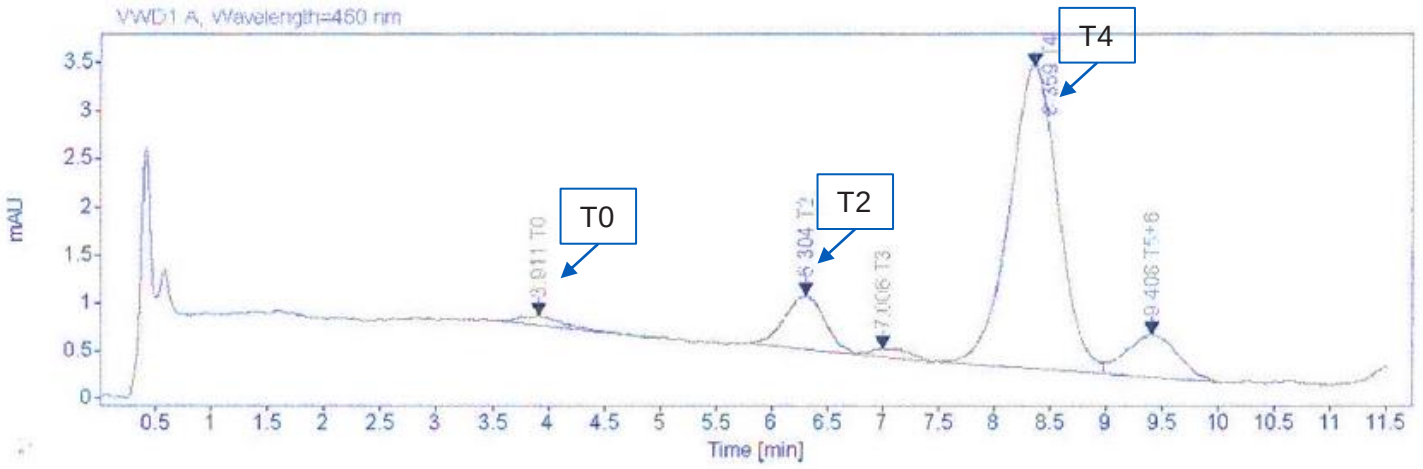
Secondary causes of an abnormal transferrin pattern:

- Galactosaemia
- Fructosaemia
- Liver disease
- Alcoholism

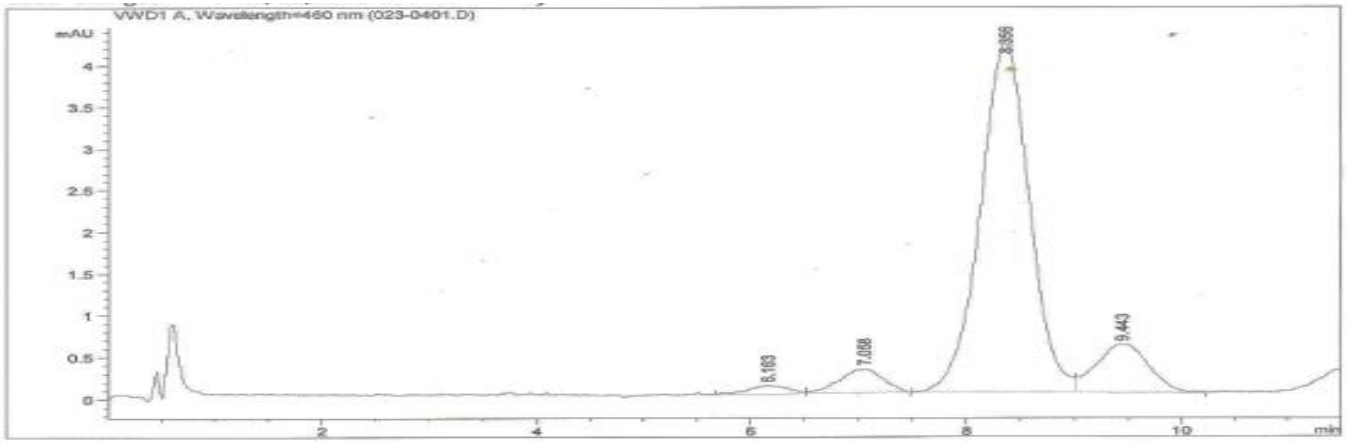
Babies <3 weeks old- maternal isoforms may mask an abnormality

This test is Transferrin Isoforms for Congenital Disorders of Glycosylation (Not for Alcoholism and Not Iron Studies!)

Case presentation



Type 1 pattern (increased T2 and T0)



Normal