Abnormal transferrin isoform profiles when screening N-linked congenital disorders of glycosylation by time-of-flight mass spectrometry

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Introduction

- Abnormal transferrin isoform patterns are a marker for N-linked congenital disorders of glycosylation (CDG)
- CDG encompass a large group of inborn errors of metabolism characterised by defective glycosylation
- Transferrin has predominantly two complex bi-antennary N-linked oligosaccharide chains
- Depending on the biosynthesis defect abnormal type I, type II or mixed type transferrin isoform patterns can be observed

Fig 1: Schematic illustrating the N-linked oligosaccharide structure of transferrin. The Di-Oligo isoform (tetra-sialo) is depicted, with two complex bi-antennary chains attached to transferrin.
Methods

Sample Preparation

- 5 µL serum is reduced with 50 mM ammonium bicarbonate, 30 mM dithiothreitol (2 h, 45°C)

Key Equipment

- Agilent 6230 time-of-flight (TOF) mass spectrometer
- Agilent 1260 binary pump
- Agilent Poroshell 300SB C3 (2.1 × 75 mm) LC column

Results

- We have detected abnormal transferrin isoform patterns by TOF mass spectrometry for PMM2-CDG, MPI-CDG, MAN1B1-CDG and SLC37A4-CDG, as well as secondary causes such as alcohol abuse and galactosaemia

PMM2-CDG

Fig 2: Abnormal type I transferrin isoform pattern detected for a patient with PMM2-CDG. This pattern is characterised by elevated A-Oligo (a-sialo) and Mono-Oligo (di-sialo) transferrin isoforms
Fig 3: Abnormal type II transferrin isoform pattern detected for a patient with MAN1B1-CDG. This diagnostic pattern is characterised by elevated tri-sialo transferrin isoforms with additional mannose attached.

Fig 4: Abnormal type I transferrin isoform pattern detected for a patient with galactosaemia. Galactosaemia is a secondary cause of an abnormal type I transferrin isoform pattern.
Conclusion

- Detection of transferrin isoforms by TOF mass spectrometry is an effective screening method for CDG and can reveal diagnostic profiles that other screening techniques can’t.
- Routine screening methods assess transferrin isoforms based on the number of terminal sialic acid residues attached to transferrin (a-sialo, di-sialo, tri-sialo, tetra-sialo, penta-sialo) and may be subject to interference from transferrin variants, or co-migrating serum proteins such as clonal immunoglobulins.
- TOF mass spectrometry can be used to identify the presence of transferrin variants and is not subject to the same interference, so is superior for screening CDG.

Acknowledgement

- Abnormal serum samples from patients with MAN1B1-CDG and SLC37A4-CDG were obtained as part of the ERNDIM CDG EQA scheme.