B-03

EXPLORATORY APPROACH OF SERUM N-GLYCAN PROFILING BY MASS SPECTROMETRY

Salina Abdul Rahman*[1], Siti Aishah Abdul Wahab[2] and Ngu Lock Hock[3]

[1]Inborn Errors of Metabolism & Genetics Unit, (NMCRC), [2]Molecular Diagnostic Protein Unit, (SDC), Institute for Medical Research, National Institute of Health Complex, Setia Alam, 40170, Shah Alam, Selangor [3]Institute of Paediatric, Jalan Pahang, 50588, Wilayah Persekutuan Kuala Lumpur, Malaysia

INSTITUTE FOR MEDICAL RESEARCH MALAYSIA

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INTRODUCTION

- Congenital disorders of glycosylation (CDG)[1] are among the inherited metabolic diseases caused by a defect in protein glycosylation.
- We have used our modified FASP[2] protocol ('glycoFASP') to determine the structures of normal and CDG Type 1 patient *N*-glycans.

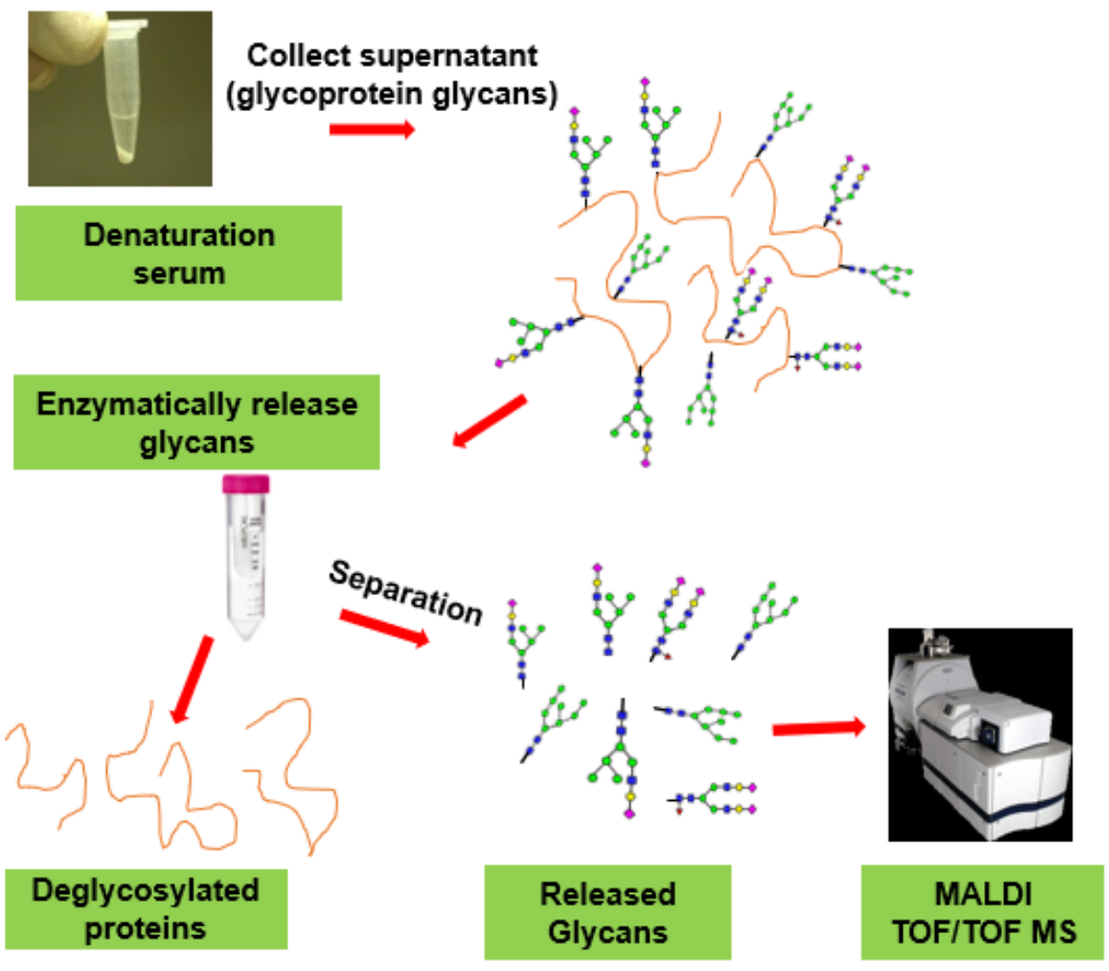
OBJECTIVES

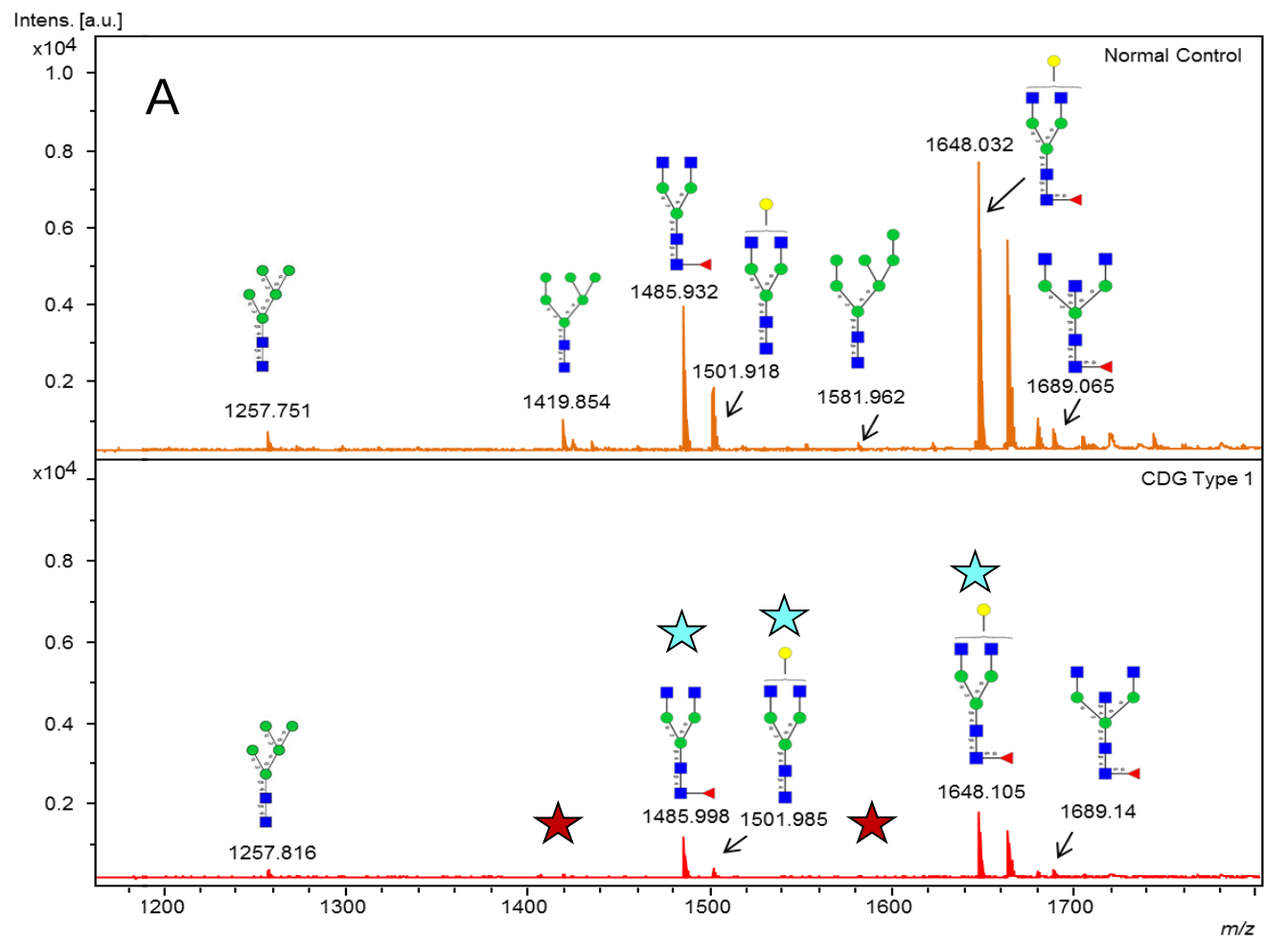
- Develop a simple FANGS[3] protocol to isolate and analyze glycoprotein glycans from human serum.
- Apply the glycoFASP method to compare *N*-glycan profiles between normal control and CDG Type 1 patient.

ADVANTAGES

- Small volume of serum needs for sample preparation
- Sensitive and easy to perform using membrane spin filters
- No requirement for sample clean up
- Avoids lengthy, multi-step procedures which can reduce *N*-glycans recovery

METHODOLOGY





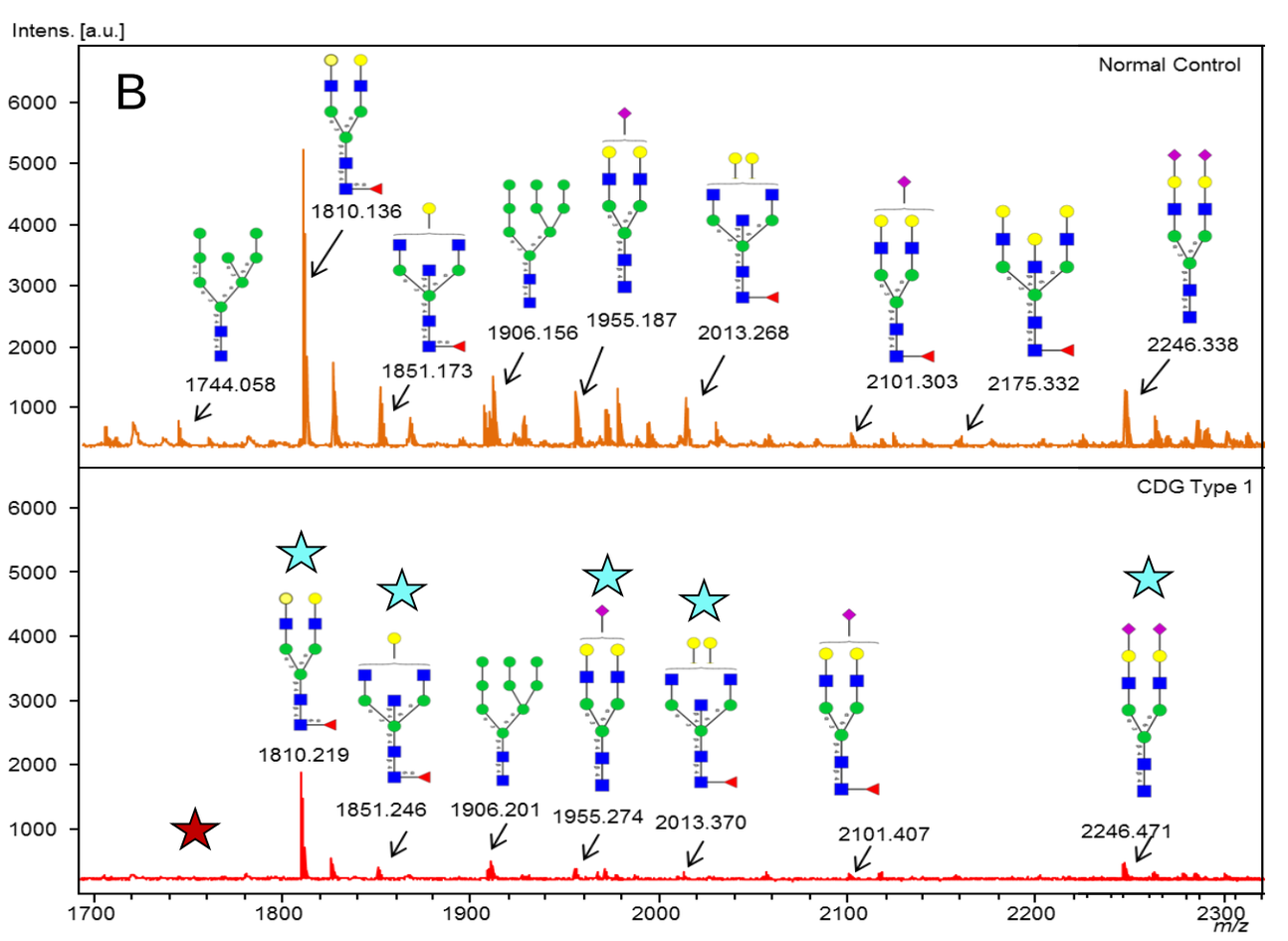


Figure 1. Spectrum of glycoFASP-released glycans from normal control and CDG Type 1 (A) low *m/z* range and (B) high *m/z* range. Legend: ■-acetyl glucosamine (GlcNac), ● Mannose (Man), ○ Galactose (Gal), ▲ Fucose (Fuc), ◆ Sialic acid (NeuAc). ★ absent, ☆ low intensity.

RESULTS

- glycoFASP-released glycans from normal control and CDG Type 1 patient were analysed using MALDI –TOF/TOF MS are shown in Figure 1.
- The spectrum shows glycans signal (*m/z* 1419, 1581 and 1744) are not observed from CDG Type 1 patient when compared to normal control spectrum.
- Normal control also produce non fucosylated (*m/z* 1501) and fucosylated (*m/z* 1485,1648 and 1810) complex bi-antennary, bisecting GlcNAc (*m/z* 1689, 1851, 2013 and 2175) and sialylated (*m/z* 1955, 2101 and 2246) glycans.
- Interestingly, CDG Type 1 spectrum also show non fucosylated (*m/z* 1501) and fucosylated (*m/z* 1485,1648 and 1810) complex bi-antennary, sialylated (*m/z* 1955,2101 and 2246) and bisecting GlcNAc (*m/z* 1689, 1851 and 2013) glycans lacking (*m/z* 2175). These *N*-glycans signal are markedly lower compared to normal control.

CONCLUSION

- The difference between normal control and CDG Type 1 glycan structures has been demonstrated.
- The protocol is less laborious and reduces time in sample preparation before MALDI analysis.
- The protocol works efficiently and convenience for low volume of sample.

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