Glycomics Profile Analysis by MALDI TOF/MS in Human CSF

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Introduction

- Genetic defects in human glycome
  - 2-3% of the human genome encodes proteins for glycosylation
  - 50% of proteins are glycosylated in human
  - 10 distinct glycoprotein biosynthesis pathways
  - >100 disease causing genes discovered
  - Many still remain unknown

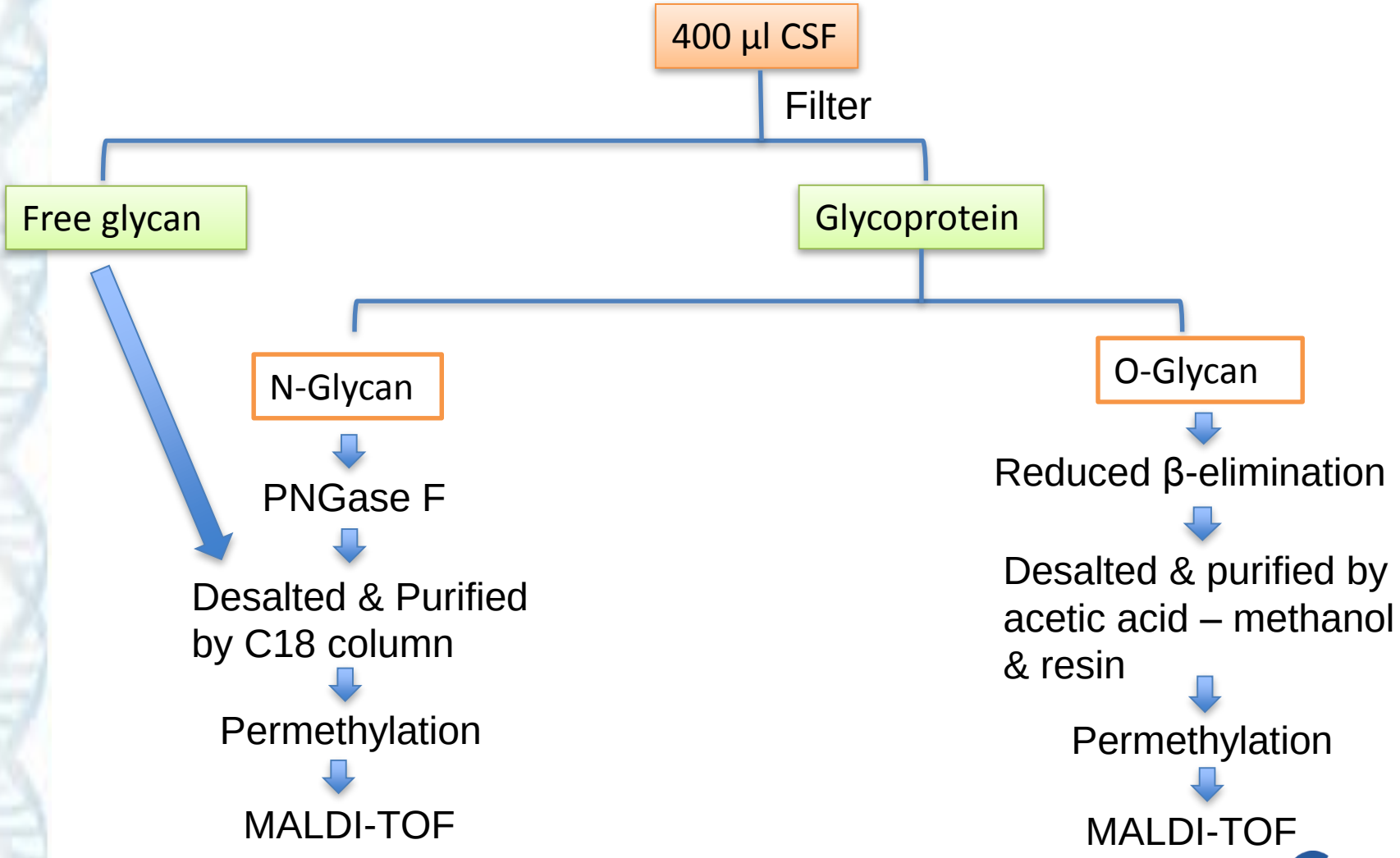
Why CSF glycome?

- Glycoproteome and glycome in plasma are limited
- Unique glycosylation type and glycogene expression pattern in brain
- CSF glycome in human has not been well studied
- Does CSF glycome provide us more diagnostic value for CDG ?

Project

- CSF free glycome
- CSF N-glycome
- CSF O-glycome
- Study cohort
  - 166 CSF from patients with undiagnosed neurological disease (NIH/UDP)
  - 10 CSF normal controls

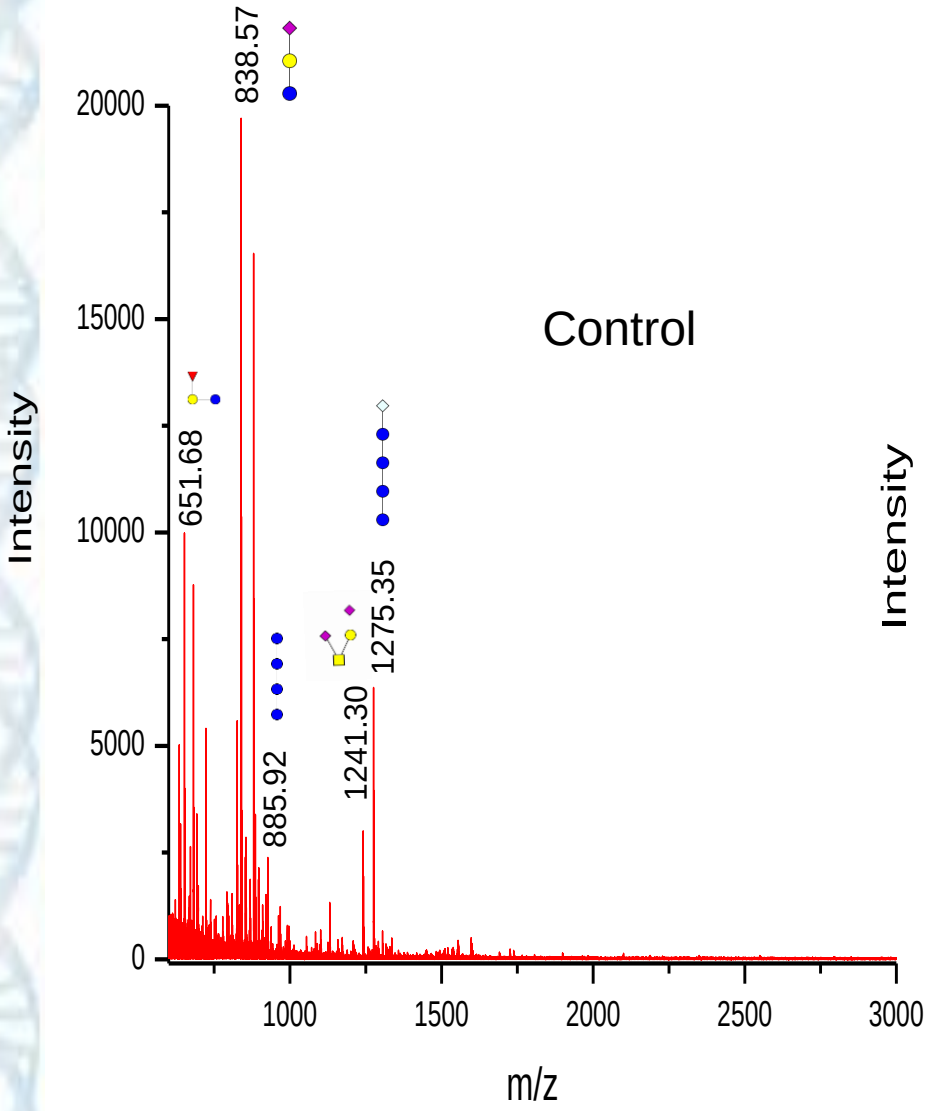
Methods

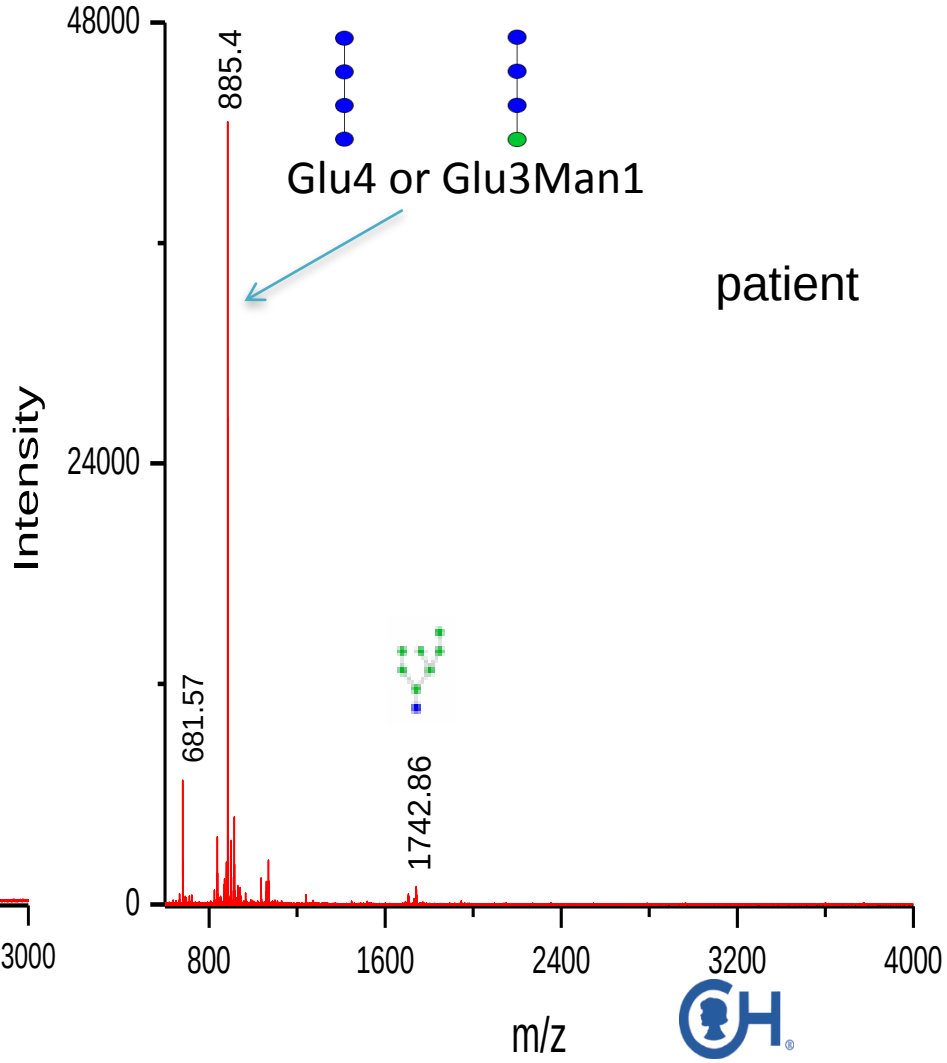

400 µl CSF
Filter
Free glycan
Glycoprotein
N-Glycan
PNGase F
Desalted & Purified by C18 column
Permethylation
MALDI-TOF
O-Glycan
Reduced β-elimination
Desalted & purified by acetic acid – methanol & resin
Permethylation
MALDI-TOF

“Undiagnosed” Cases

- Brother (MM, 11 year old)
  - Encephalopathy/CP/Spastic Paraparesis
  - Congenital Hypotonia
  - Global Developmental Delay
  - Bilateral Hearing Loss
  - Multiple Fractures
  - Recurrent Pneumonias
  - Low immunoglobulin level, low IgA
  - Generalized Aminoaciduria
  - CDG normal by CDT
- Sister (IM, 6 year old)
  - Profound Global Developmental Delay
  - Congenital Hypotonia
  - Neonatal Seizures
  - Cerebral atrophy, small corpus callosum
  - History of recurrent UTIs
  - CDG normal by CDT

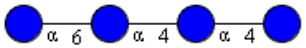
Sib's Urine Free Glycan





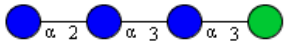
Differential For Hex4

- Pompe Disease



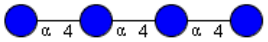
  - acid alpha-1,4-glucosidase (GAA), 17q25.2-q25.3

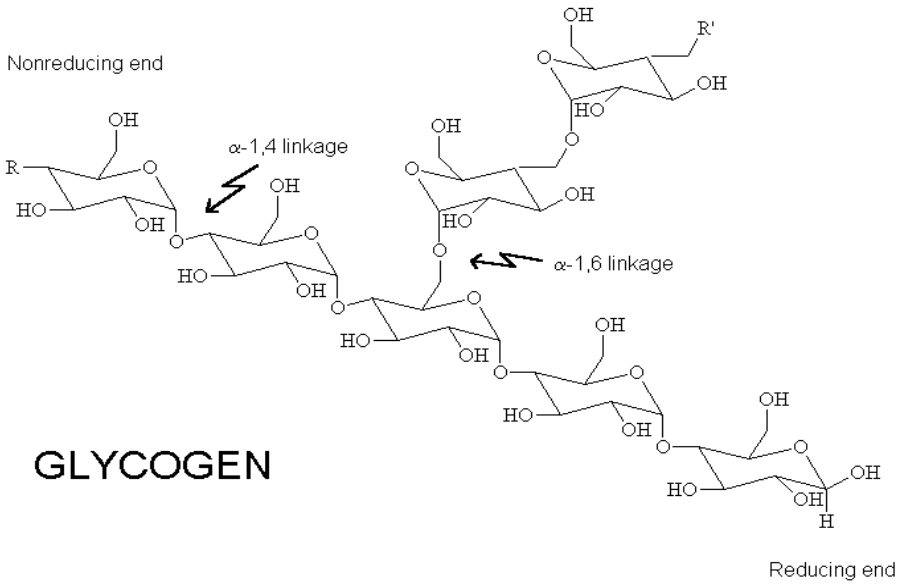
- CDG IIb

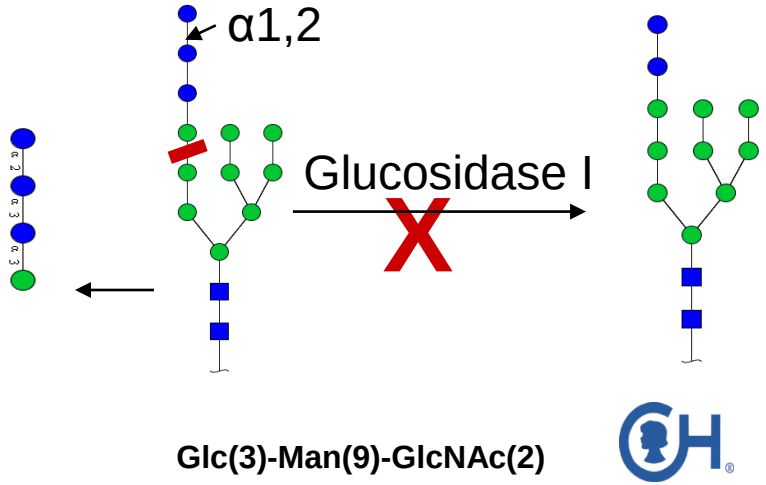


  - glucosidase I (GCS1), 2p13-p12

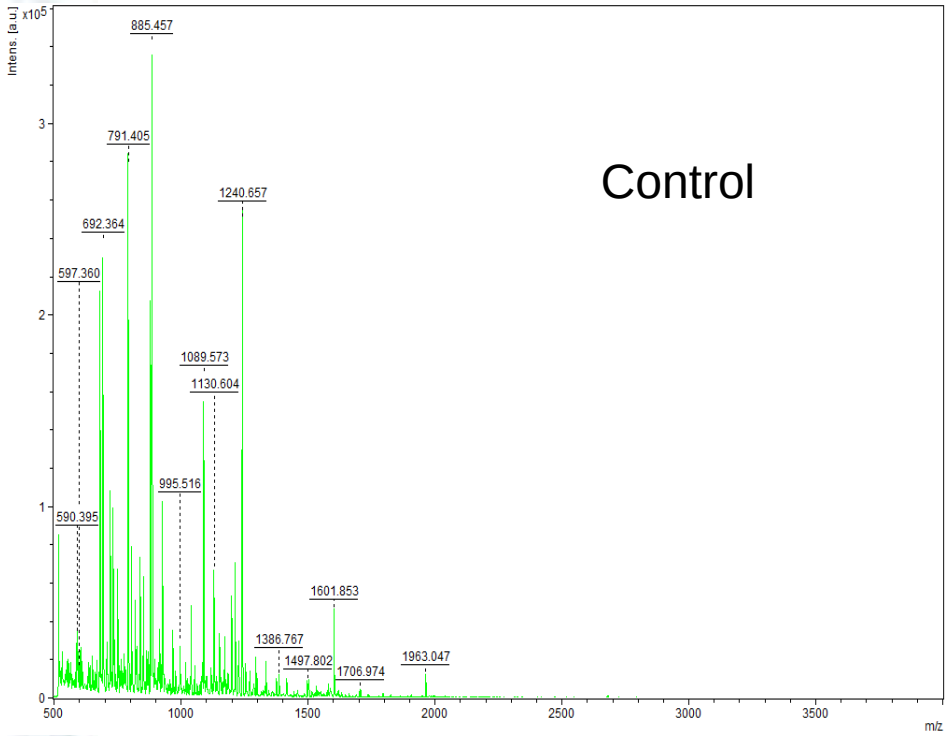
- Polyglucose Diet



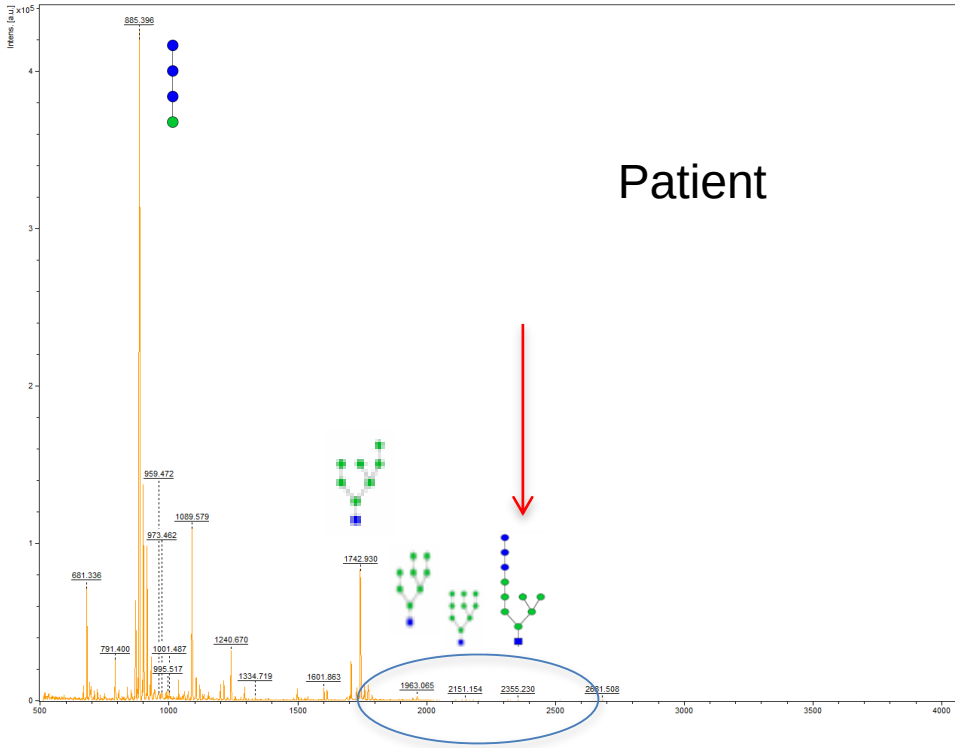




Sib's CSF Free Glycan



Control



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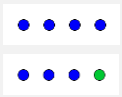
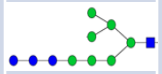
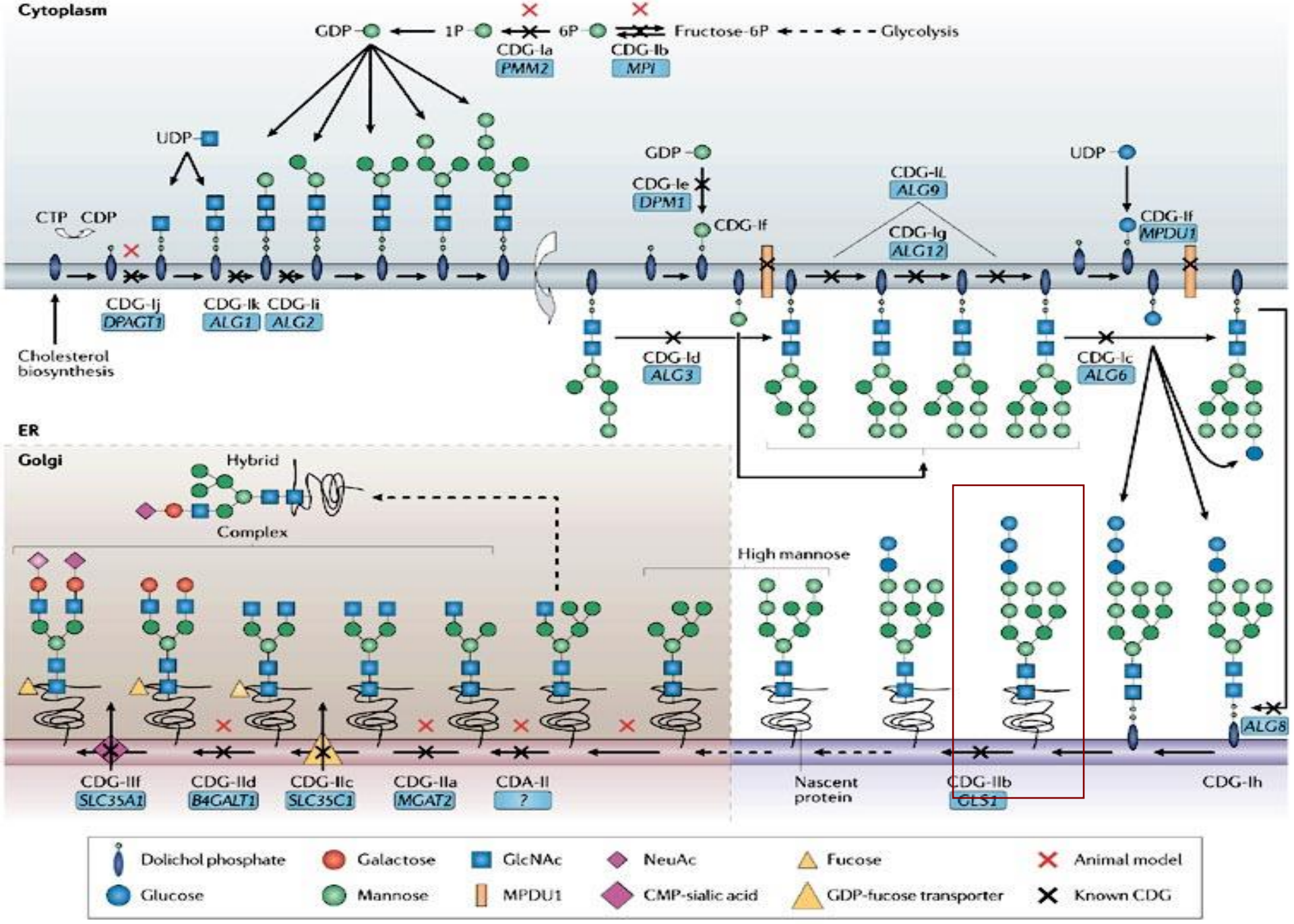
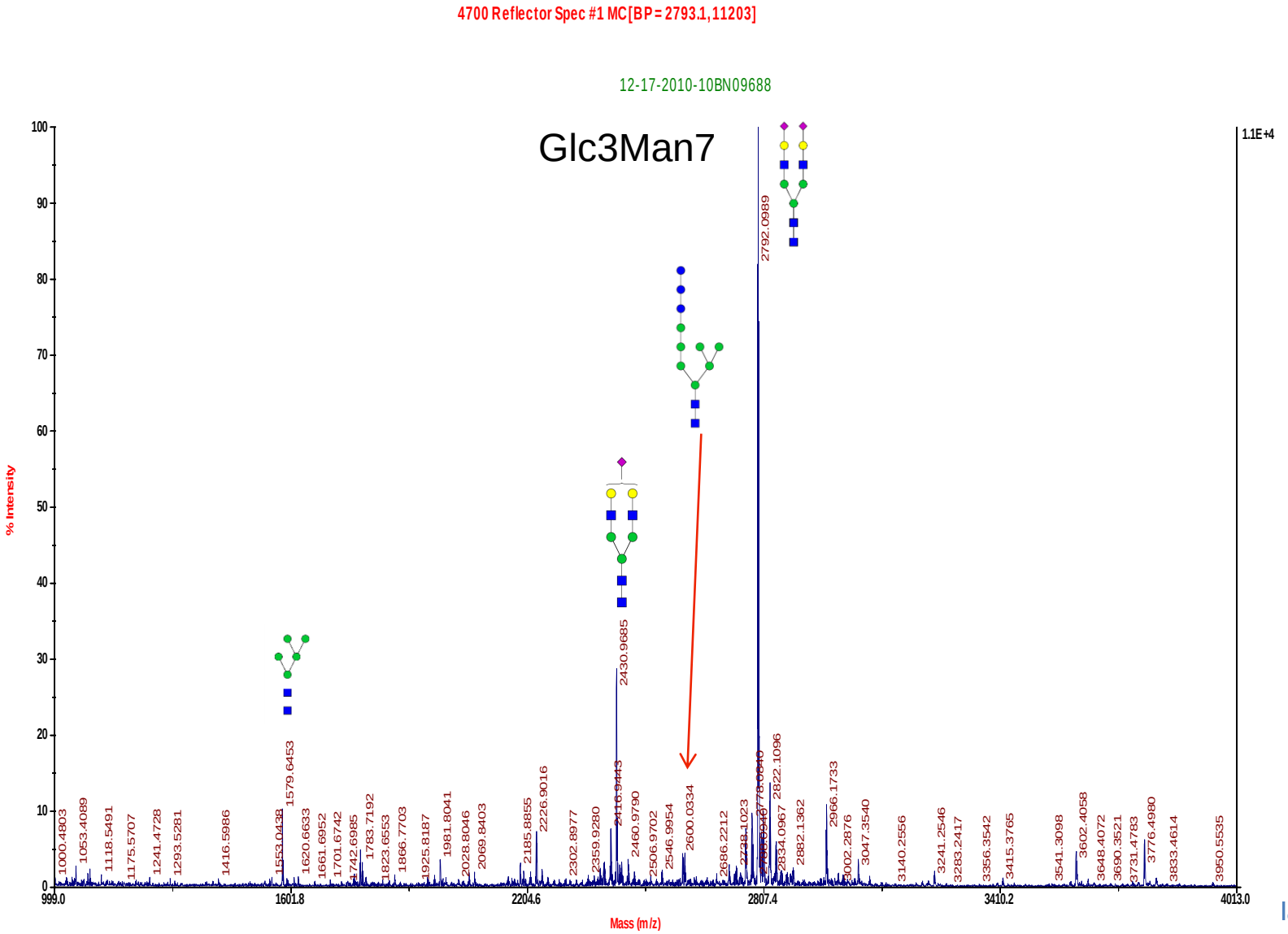
Abnormal CSF Free Glycans

m/z			value		Reference range
885.40		Hex4 (Glu4 or Glu3Man1)	43.21%	H	1.37-8.59%
1538.697		Man6 GlcNAc1 or Glu3Man3GlcNAc1	0.41%	H	0.0-0.13%
1742.86		Man7 GlcNAc1 or Glu3Man4GlcNAc1	17.07%	H	0.0-0.0%
1946.96		Man8 GlcNAc1 or Glu3Man5GlcNAc1	0.32%	H	0.0-0.0%
2151.06		Man9 GlcNAc1 or Glu3Man6GlcNAc1	0.14%	H	0.0-0.0%
2355.06		Glu3Man7GlcNAc1	0.10%	H	0.0-0.0%

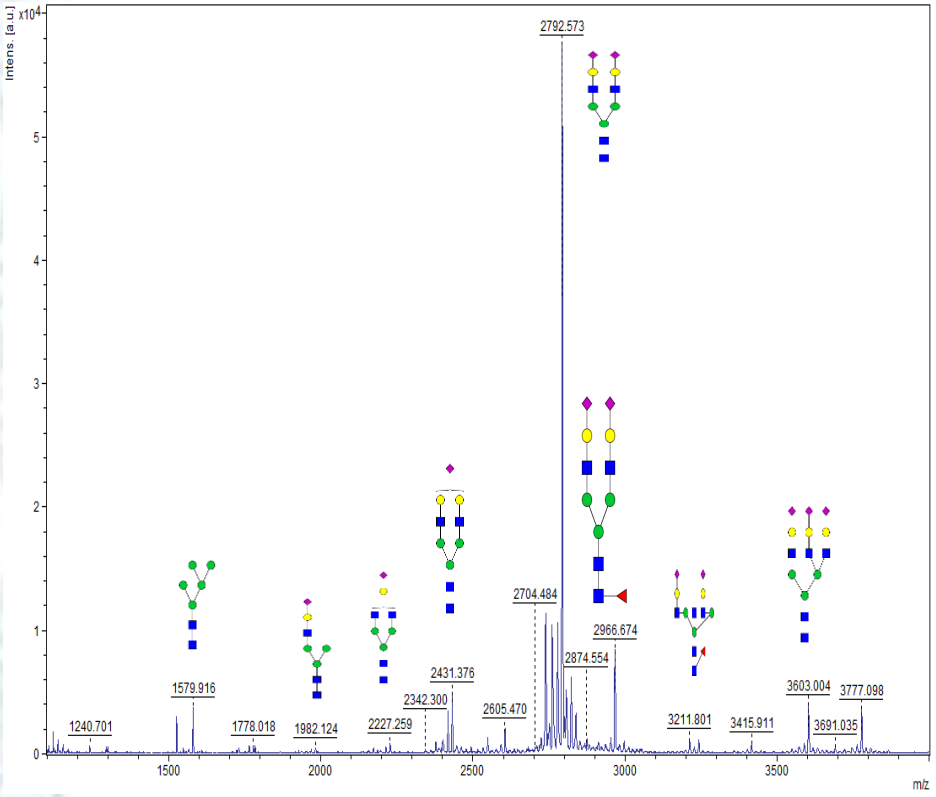


Sib's Plasma N-glycan

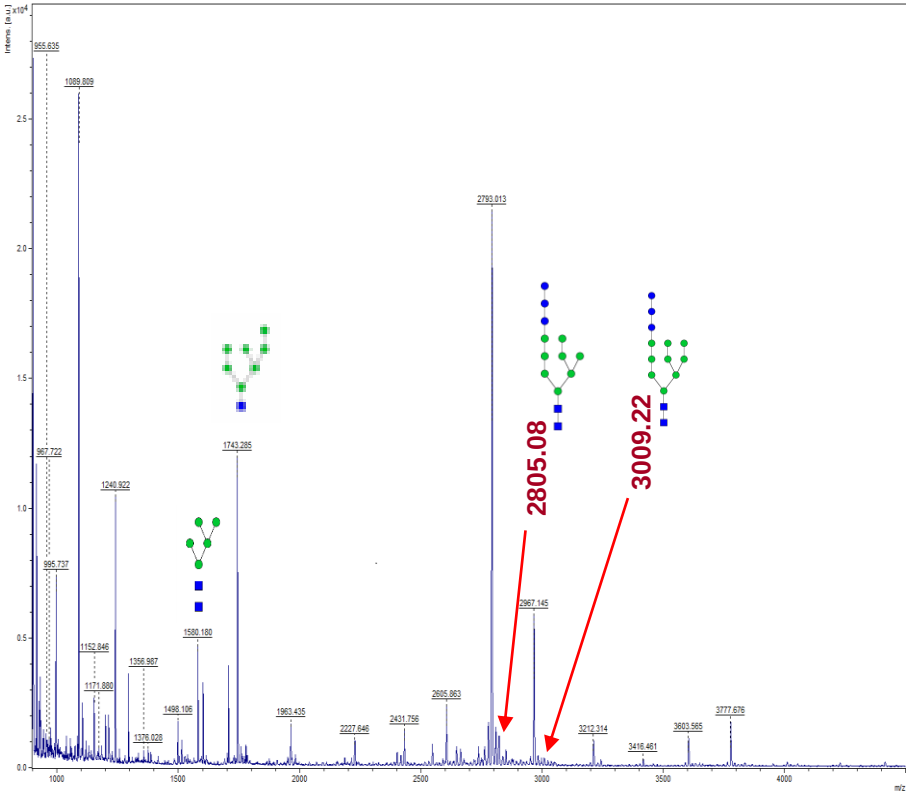


Sib's CSF N-Glycan Compare with Control

Control CSF N-Glycan



Patient CSF N-Glycan



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MOGS Seq

- Affected male sibling

  Exon 1 c.65C>A, p.A22E; c.329G>A, p.110R>H;

  Exon 2 c.370C>T, p.124Q>X

- Affected female sibling

  Exon 1 c.65C>A, p.A22E; c.329G>A, p.110R>H

  Exon 2 c.370C>T, p.124Q>X

- Mother

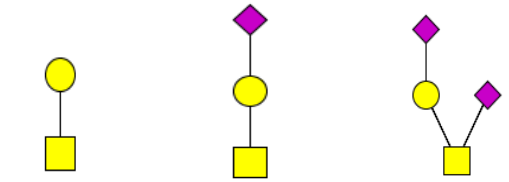
  Exon 2 c.370C>T, p.124Q>X

- Father

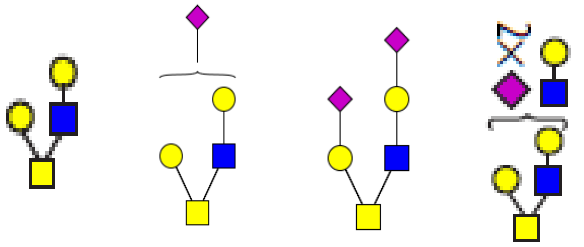
  Exon 1 c.65C>A, p.A22E; c.329G>A, p.110R>H;

GalNAc Type O-linked Protein Glycosylation

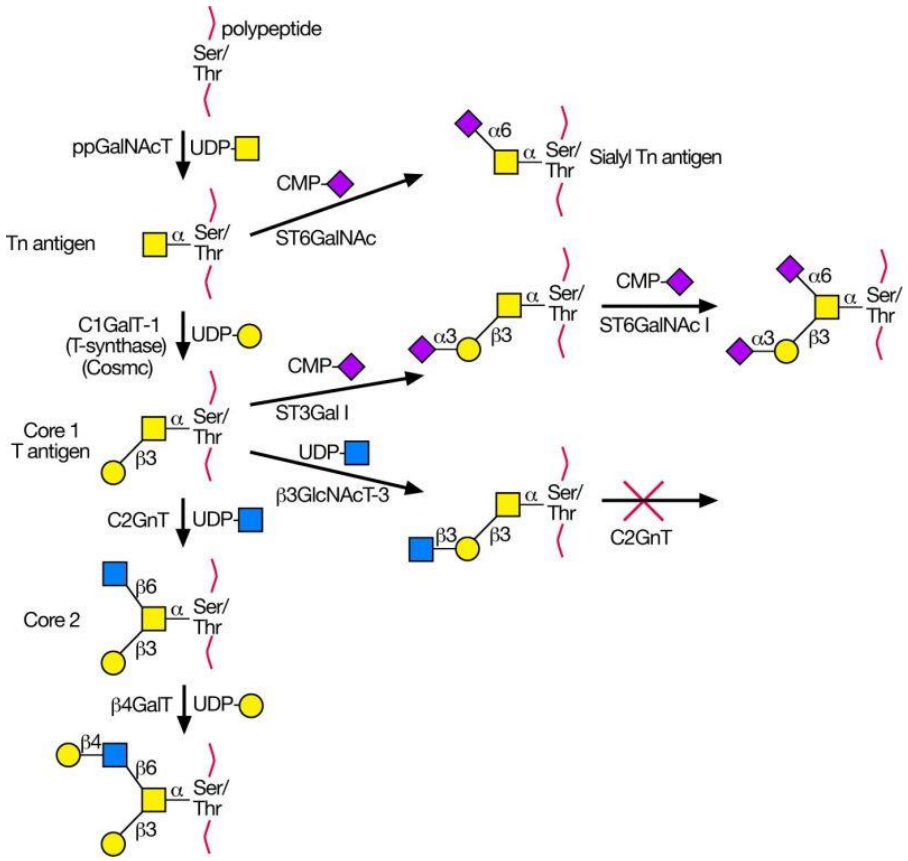
Core1



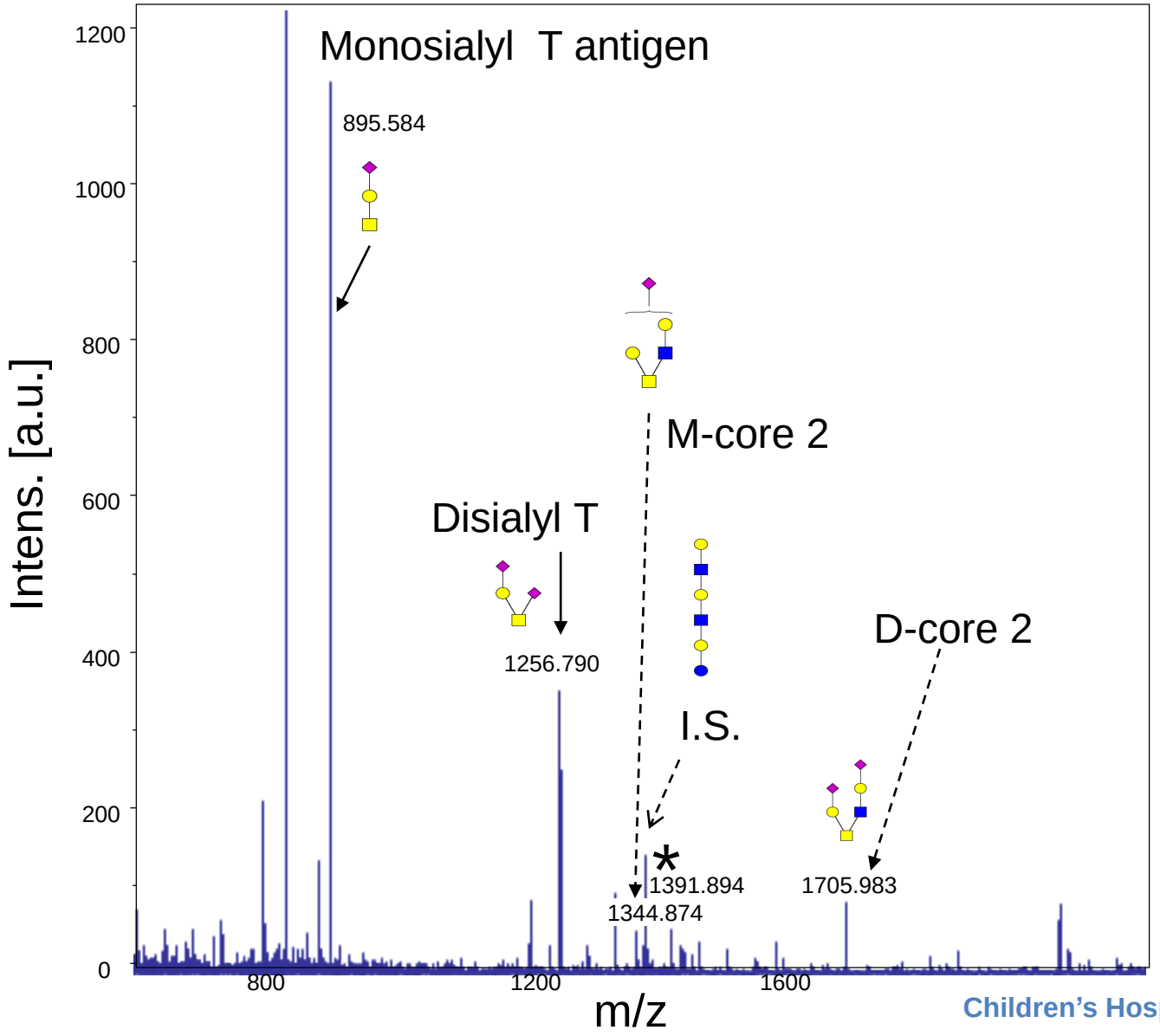
Core2



Biosynthesis of Core 1 and Core 2



Plasma O-glycan Profile



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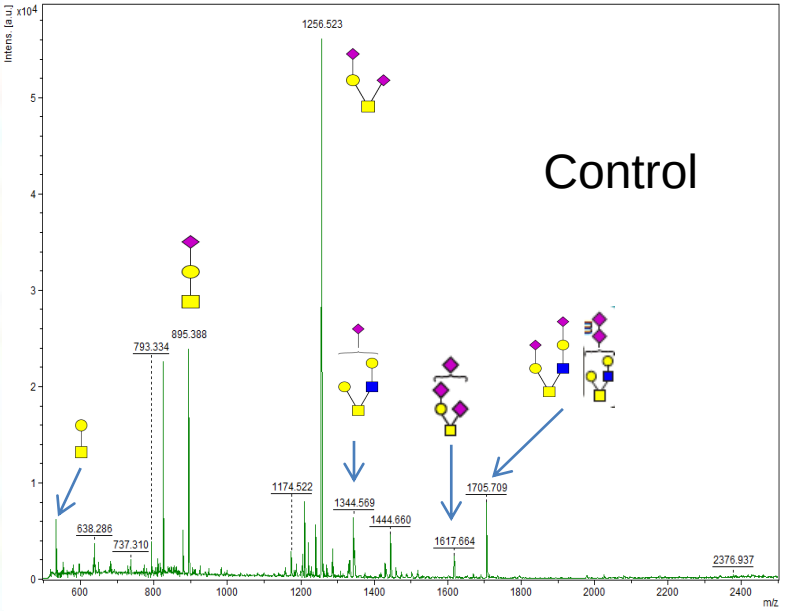
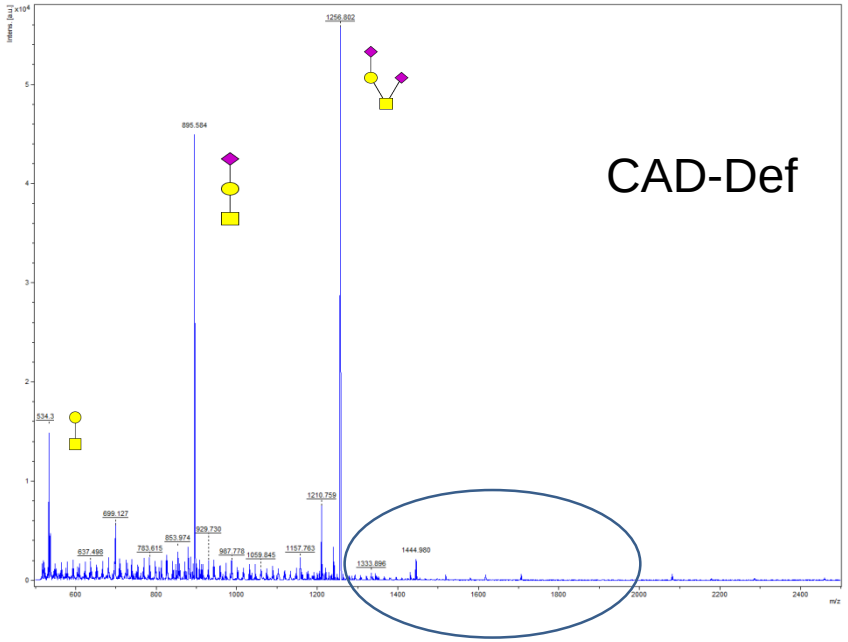
CSF O-glycan Profiles

- Control and abnormal CSF O-linked glycan





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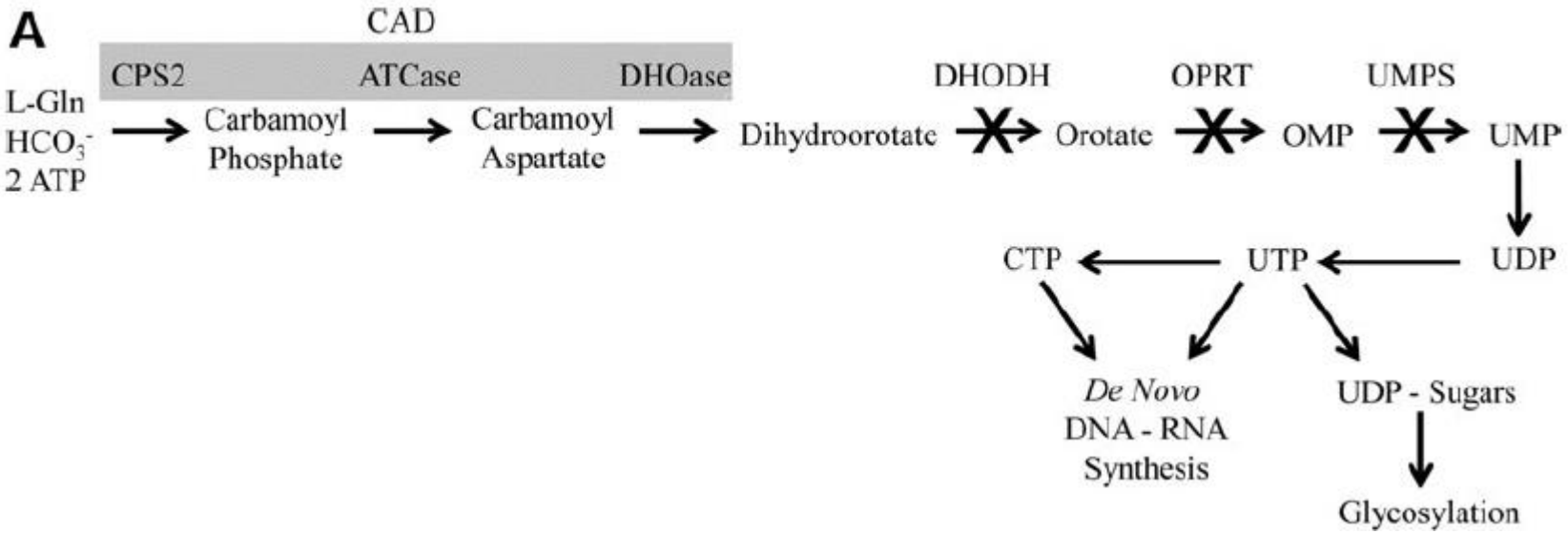
CAD Deficiency

ORIGINAL ARTICLE

Biallelic mutations in *CAD*, impair *de novo* pyrimidine biosynthesis and decrease glycosylation precursors

Bobby G. Ng[1,†], Lynne A. Wolfe[2,†], Mie Ichikawa[1], Thomas Markello[2], Miao He[4], Cynthia J. Tifft[2,3], William A. Gahl[2,3] and Hudson H. Freeze[1,*]



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Conclusion

- CSF glycome is different and more complex than plasma glycome
  - Free glycome, N-glycome and O-glycome
- CSF glycome may detect CNS-specific defect in protein glycosylation
- CSF glycome provides additional information for the diagnosis of CDG

Acknowledgment

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Bill Gahl, MD. Ph.D.

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