

Glycoanalysis

P05:



Goes Virtual (October 7- 8, 2021)

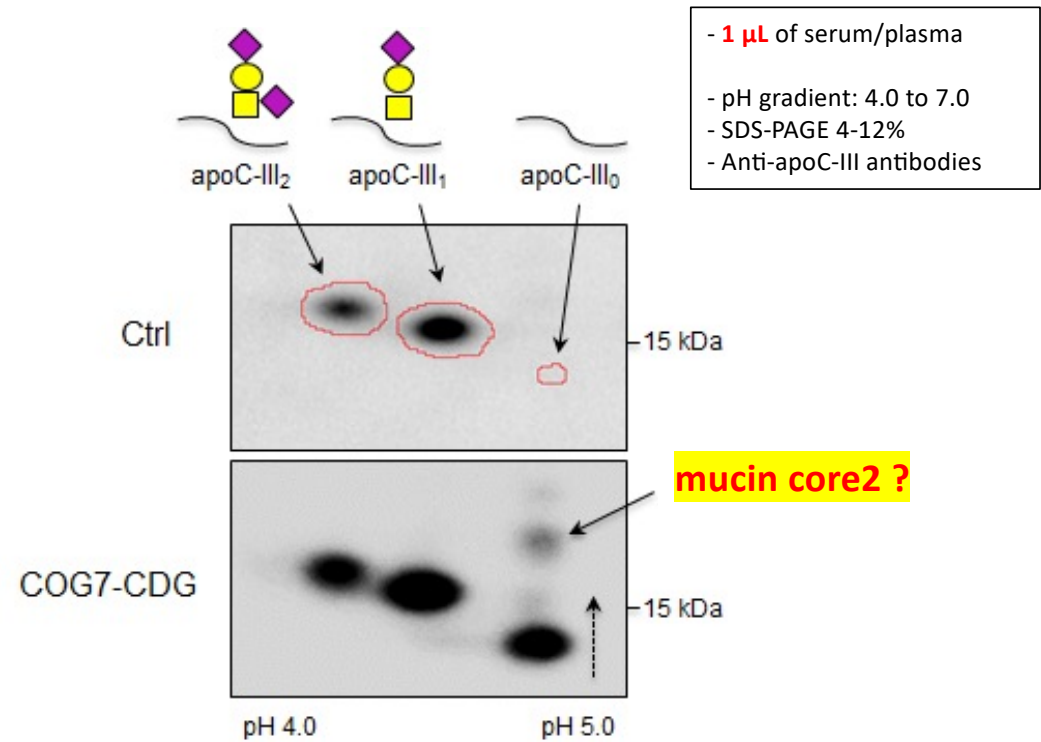
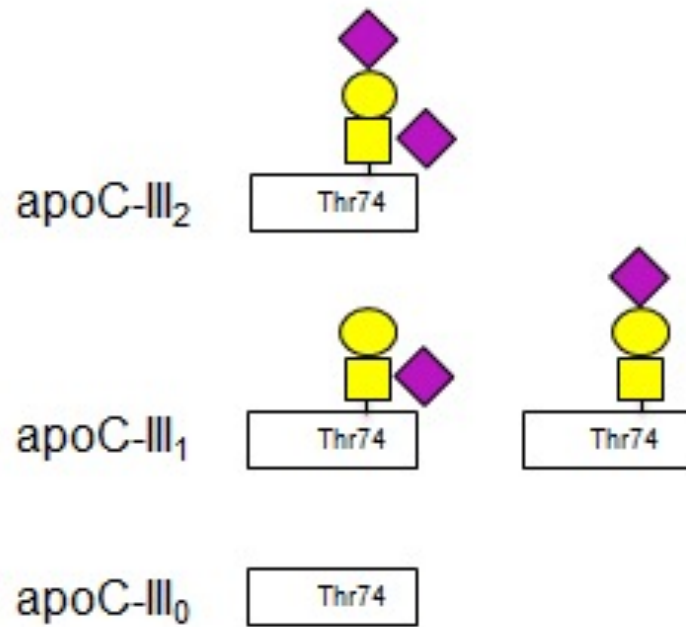
## Two-dimensional electrophoresis highlights minor mucin core2 O-linked glycoforms of serum apolipoprotein C-III

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Apolipoprotein C-III (apoC-III) is a mucin **core1** O-glycosylated protein



- 1  $\mu$ L of serum/plasma
- pH gradient: 4.0 to 7.0
- SDS-PAGE 4-12%
- Anti-apoC-III antibodies

**ApoC-III is a mucin core2 O-glycosylated protein**

**Identification of New Apolipoprotein-CIII Glycoforms with Ultrahigh Resolution MALDI-FTICR Mass Spectrometry of Human Sera**

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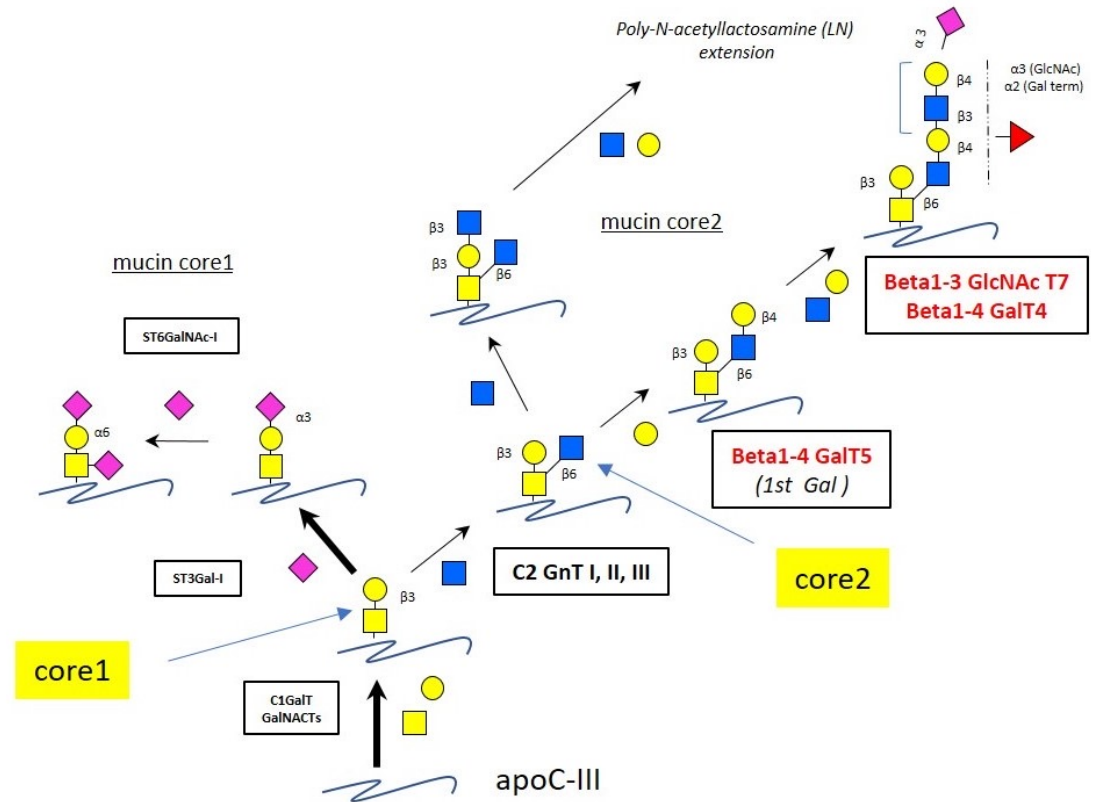
**Table 1. Isoforms of Apolipoprotein-CIII**

Apolipoprotein-CIII amino acid sequence:  
SEAEDASLLSFMQGYMKHATKTAKDALSSVQESQVAQ-  
QARGWVTDGFSLLKDYWSTVKDKFSEFWDLDPVVRPTSAVAA

observed apolipoprotein-CIII isoform	calculated $m/z$ -value [M + H] <sup>+</sup>	frequency in 96 serum samples
apoCIII <sub>0a</sub> (-)	8765.2385	100%
apoCIII <sub>0c</sub> (HexHexNAc)	9130.3708	100%
apoCIII <sub>1</sub> (HexHexNAcNeuAc) <sup>a</sup>	9421.4663	100%
apoCIII <sub>2</sub> (HexHexNAcNeuAc) <sup>b</sup>	9712.5618	100%
apoCIII-Hex <sub>2</sub> HexNAc <sub>2</sub> Fuc <sub>3</sub> <sup>b,c</sup>	9933.6769	27%
apoCIII-Hex <sub>3</sub> HexNAc <sub>3</sub> Fuc <sub>2</sub>	10153.7538	11%
apoCIII-Hex <sub>4</sub> HexNAc <sub>3</sub> Fuc <sub>3</sub>	10258.7852	8%
apoCIII-Hex <sub>2</sub> HexNAc <sub>4</sub> Fuc <sub>3</sub>	10340.8383	29%
apoCIII-Hex <sub>3</sub> HexNAc <sub>3</sub> Fuc <sub>4</sub>	10445.8698	12%
apoCIII-Hex <sub>3</sub> HexNAc <sub>3</sub> Fuc <sub>4</sub>	10852.0286	6%

<sup>a</sup>Structure confirmed with ESI-FTICR-MS/MS in a previous study.<sup>25</sup>

<sup>b</sup>Structure confirmed with ESI-FTICR-MS/MS. <sup>c</sup>Structure confirmed with LC-IT-MS/MS.

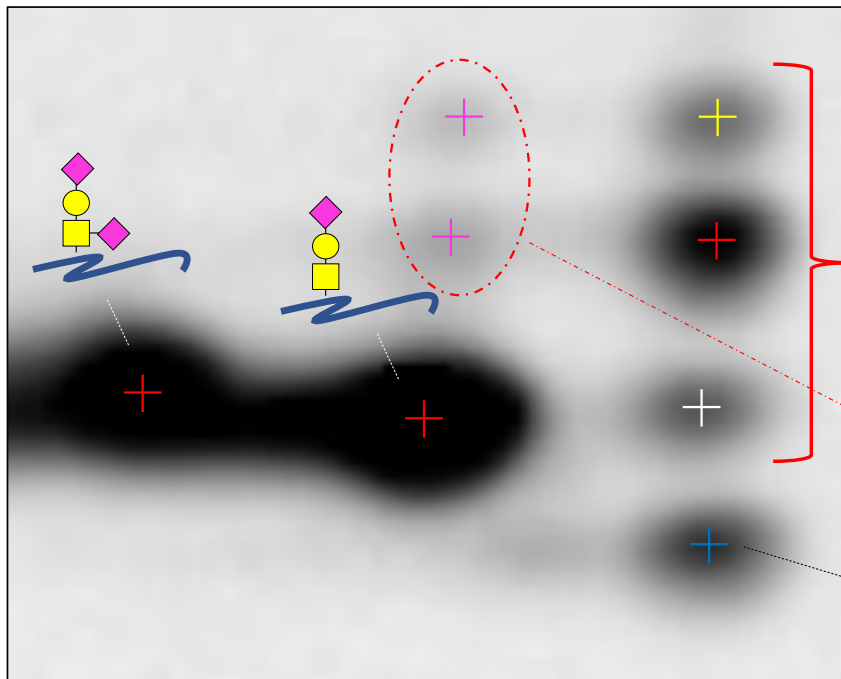




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ApoC-III is a mucin **core2** O-glycosylated protein

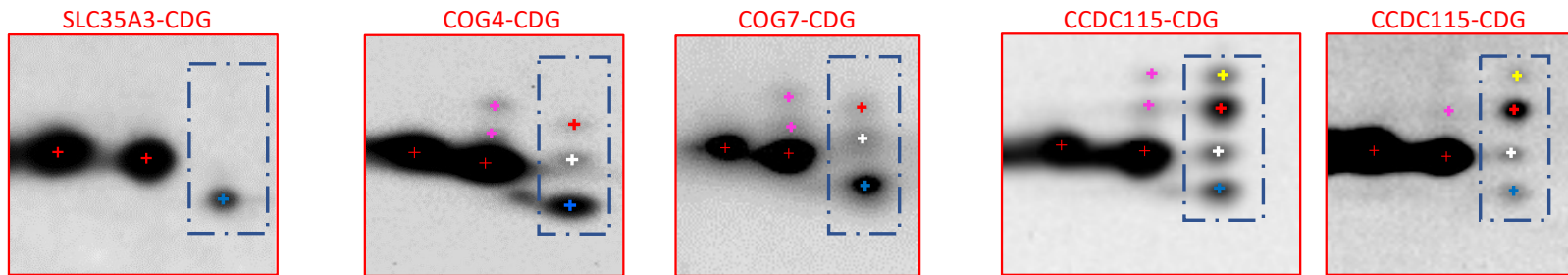
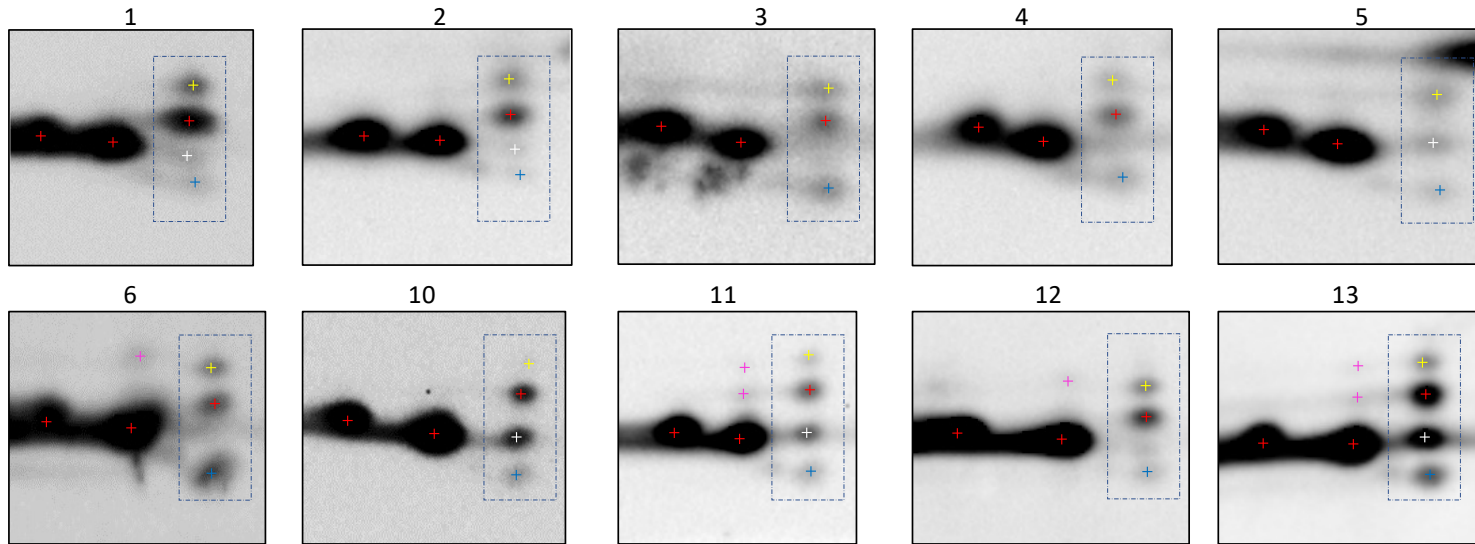
- 10  $\mu$ L of serum/plasma
- pH gradient: 4.0 to 7.0
- SDS-PAGE 4-12%



Probable asialylated and +/- fucosylated mucin core2 apoC-III glycoforms

Probable monosialylated and +/- fucosylated mucin core2 apoC-III glycoforms

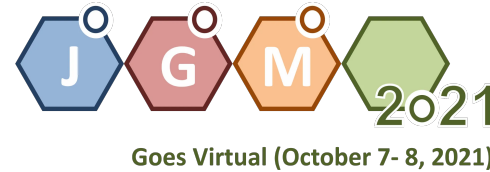
Mucin core2 apoC-III O-glycoforms in controls and some CDG



UDP- GlcNAc transporter deficiency

Traffic homeostasis (cis-Golgi) deficiency

pH homeostasis (trans-Golgi) deficiency



## Conclusions and perspectives

- 2-DE allows the separation and relative quantification of highly probable mucin core2 apoC-III glycoforms
- Probable overestimation of % mucin core1 apoC-III<sub>0</sub> with IEF alone
- Heterogeneous profiles in controls
- Undetectable mucin core2 glycoforms in SL35A3-CDG (UDP-GlcNAc transporter)
- Signature profiles in COG-CDG and in CCDC115-CDG ??
  
- Additional controls and CDG samples will be analyzed
- Characterization of mucin core2 glycoforms of interest (lectins / enzymes / MS...)
- Balanced regulation of mucin core1 / mucin core2 glycoforms in CDG?
- Potential biomarkers of fucosylation defects?

**THANK YOU!**

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