# Apolipoprotein C-III mucin core2 O-linked glycoforms: hiding in plain sight

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## INTRODUCTION

Glycosylation is a covalent attachment of a polysaccharidic chain to a protein or a lipid, generally catalyzed by glycosyltransferases, using specific nucleotide sugar donors. N-glycosylation is the best known and major form of glycosylation. However, there is also O-glycosylation type that is still not well-known and on which we focused in this work.
Mucins are the class of glycoproteins carrying the greatest number of mucin-type O-glycans. They have multiple effects on the immune system and maintenance of cellular homeostasis. We specifically focused on apolipoprotein C-III (apoC-III) which is a serum mucin core1 and core2 O-glycosylated protein (**Fig. 1**). We qualified mucin core2 glycoforms of apoC-III by employing two-dimensional electrophoresis.

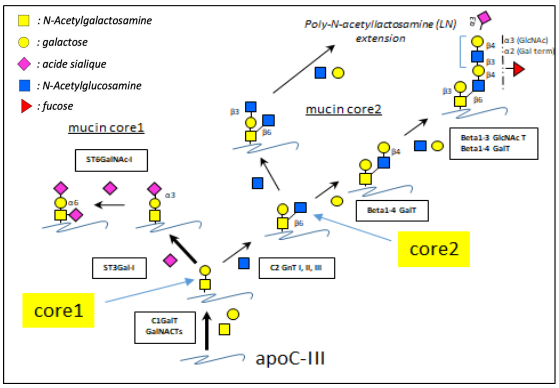


**Figure 1**: Mucin-type O-glycosylation pathways (ref 1)

## MATERIALS AND METHODS

We chose 11 serum samples of patients with normal transferrin profile as controls and 7 serum samples of patients with various congenital disorders of glycosylation (CDG):
- SLC35A3-CDG (n=1) : default of Golgi UDP-N-acetylglucosamine (GlcNac) transporter.
- CCDC115-CDG (n=2) : Golgi pH alkalinization
- SLC37A4-CDG (n=2) : Golgi pH acidification
- COG-CDG (n=2) : Golgi trafficking defect

Two-dimensional electrophoresis (2-DE) coupled to western blotting was performed using 10 µL of sample. Isoelectrofocalisation was performed on a ZOOM strip (pH 4-7) and SDS-PAGE was performed on a NuPAGE Bis Tris gel (4-12%). Proteins were transferred onto nitrocellulose and membranes were incubated in TTBS - 5% milk with a rabbit primary antibody (anti-apoC-III, 1/5000 v/v; BioDesign international) and then with a secondary antibody (1/5000 v/v, GE Healthcare). Revelation was performed using Clarity ECL reagent and profiles were acquired with an XRS Chemidoc camera (Bio-Rad).

## RESULTS

**Fig. 2A:** 2-DE shows a typical pattern of apoC-III core2 O-glycoforms in controls : 3 spots which, as previously described (ref 2), represent different asialylated glycoforms with variable mass and intensity.
**Fig. 2B:** In SLC35A3-CDG (Golgi UDP-GlcNAc transporter deficiency), it can be noted the absence of any spot. Since GlcNac is crucially involved in the initiation of the mucin core2 pathway, this is an indication that spots of interest are indeed very probably core2 O-linked glycoforms.

Contrary to mucin core1 pathway which is known to be impacted in CCDC115-CDCDG (ref 3), mucin core2 pathway appears to be fully functional with similar profile to that of the control.
In COG-CDG, it can be observed the accumulation of unglycosylated apo-CIII. Nevertheless, despite the early blocage, it seems that mucin core2 pathway is still functional given the presence of additional core2 glycoforms.
Finally, in SLC37A4-CDG, mucin core2 glycosylation seems to be affected at an intermediate stage of the biosynthesis process (red arrow).

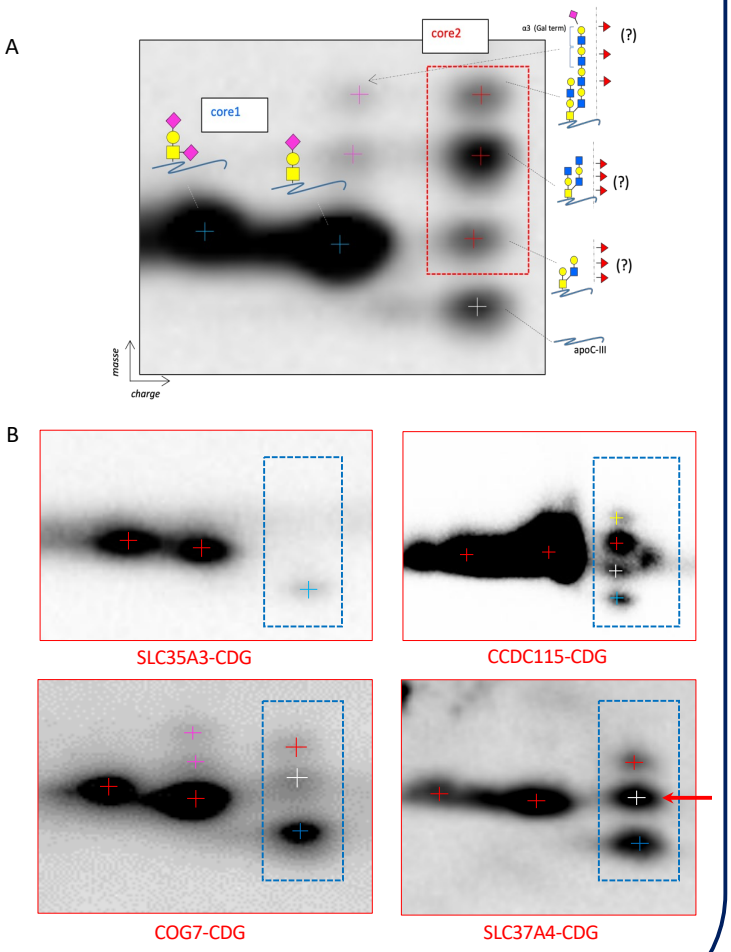


**Figure 2**: Two-dimensional electrophoresis profiles of control with normal transferrin profile (A) and CDG patients (B)

## CONCLUSION

2-DE has been developped to qualify mucin core2 O-glycoforms of apoC-III that correspond to largely unexplored and important glycosylation pathway. This promising technique may be a simple and efficient tool in CDG diagnosis and fundamental understanding with potentials as a source of novel biomarkers in acquired diseases such as hepatopathies.

## REFERENCES

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