# Proteoglycomics tools applied to the screening and characterization of combined congenital disorders of glycosylation: when “omics” and “clinics” are going together

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**CONTEXT:** In the field of congenital disorders of glycosylation (CDG), diseases combining abnormalities in both O- and N-glycosylation of proteins constitute arising novel entities. Defects in subunits of the conserved oligomeric Golgi (COG) protein complex have been shown to be involved in an important part of previously unsolved combined CDG; furthermore, recent studies revealed that autosomal recessive cutis laxa type II (a rare cutaneous congenital disease) could also be associated with such glycosylation defects in relation with mutations in the *ATP6V0A2* gene encoding the α2 subunit of the $V_0$-$H^+$ ATPase protein.

**SAMPLES:**
More than 300 serum samples from patients with clinical suspicion of CDG were screened for *O*- and *N*-glycan biosynthesis defects.

**METHODS:**
**→ Two dimensional gel electrophoresis (2-DE) and Western-blot**
- *N*-glycoproteins: Tranferrin
- *O*-glycoproteins: apolipoprotein C-III (apoC-III)

**→ Mass spectrometry** (MALDI-TOF)
- *N*- and *O*-glycans released from serum glycoproteins
- PNGase F digestion for *N*-glycans
- reductive β-elimination for *O*-glycans
-derivatization by permethylation

**→ *ATP6V0A2* DNA sequencing**

**RESULTS:**
By comparison with controls (normal % values: apoC-$III_0$ < 5%; apoC-$III_1$ : 40-75%; apoC-$III_2$: 25-60%, Fig.1-A), 2-DE of apoC-III showed abnormalities in O-glycans from five sera: two with “apoC-$III_0$” patterns (increased % of the asialylated apoC-III isoforms, Fig.1-B); one with “apoC-$III_1$” pattern (increased % of the monosialylated isoform, Fig. 1-C); two with “apoC-$III_2$” patterns (increase % of the bi-sialylated isoform, Fig. 1D). 2-DE of transferrin also showed N-glycans abnormalities (CDG-IIx) in patients with apoC-$III_0$ and apoC-$III_1$ patterns (Fig. 2). All glycomic data were corroborated at the overall level using MALDI-TOF MS of *O*- and *N*-glycans released from serum glycoproteins (Fig.3). DNA sequencing of *ATP6V0A2* in the patient with apoC-$III_1$ pattern (and with cutis laxa) showed an homozygous G deletion leading to a stop codon (Fig.4) and which was retrieved at the heterozygous state in the parents. Furthermore, patients with apoC-$III_0$ patterns are clinically highly suggestive of COG subunits congenital defects.

**CONCLUSION:**
When combined with clinical findings, 2-DE of apoC-III and transferrin coupled to MALDI-TOF MS analysis of serum glycans are powerful and complementary tools for the screening and the characterization of CDG combining *N*- and *O*-glycans biosynthesis defects.

**Figure 1:** 2-DE of apoC-III

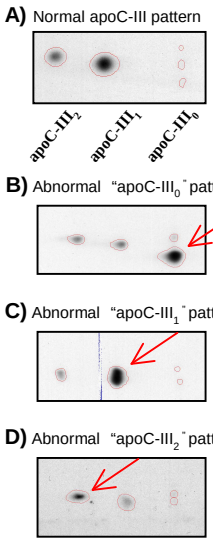


**Figure 2:** 2-DE of Transferrin

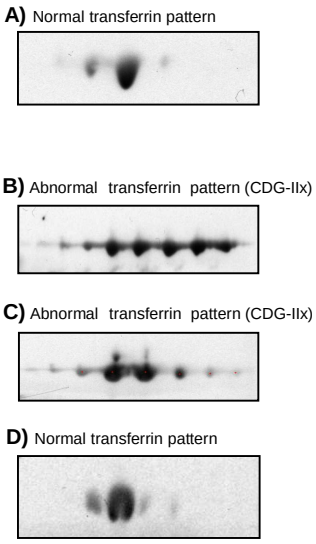


**Figure 3:** MALDI-TOF MS of serum *N*- (left) and *O*-glycans (right) from patient with apoC-$III_1$ pattern and CDG-IIx transferrin pattern

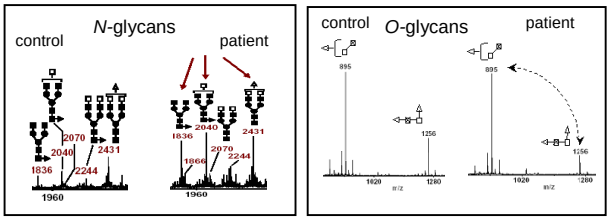


**Figure 4:** *ATP6V0A2* DNA sequencing (patient with apoC-$III_1$ pattern)