MALDI-TOF MS analysis of serum N-glycans and glycoproteins: an essential analytical tool in the diagnosis scheme of congenital disorders of glycosylation

Arnaud Bruneel[a] Sophie Cholet[b] Coralie Ruel[b], Stéphanie Yen-Nicolaÿ[c], Nathalie Seta[a], **François Fenaille**[b]

•*a: AP-HP, Biochemistry, Bichat Hospital, Paris, France; b: CEA, UMR 0496, Laboratoire d'Etude du Métabolisme des Médicaments, Université Paris-Saclay, France; c:* Trans-Prot, Proteomic facility, Université Paris-Sud, IFR141 IPSIT.

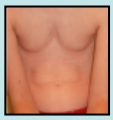
INTRODUCTION

Congenital disorders of glycosylation (CDGs) are rare autosomic recessive diseases sharing very diverse clinical symptoms. CDGs with abnormal protein N-glycosylation are sub-grouped as type I (CDG-I) or type II (CDG-II), according to transferrin (Trf) glycoforms patterns. In CDG-I, the defect alters the oligosaccharide synthesis/transfer to proteins leading to partial non-occupancy of N-glycosylation sites. In CDG-II, the defect alters the maturation of protein-linked oligosaccharide in the Golgi apparatus with alterations of N-glycans motifs. MALDI-TOF MS profiling of serum N-glycans (Goyallon *et al.*, 2015) is regarded as a powerful tool for the characterization of CDG-II related N-glycosylation defects. Furthermore, we also showed that MALDI-TOF MS of serum apolipoprotein C-III (apoC-III) can be used to screen for associated mucin core1 O-glycosylation defects (Yen-Nicolaÿ *et al.* 2015). In an integrated view also implying clinical evaluation, capillary electrophoresis of Trf, gene sequencing and whole exome sequencing data, we show here that MALDI-TOF MS can greatly help diagnosing CDGs by accurately characterizing abnormal glycan structures thereby pinpointing deficient proteins and related gene mutations.

RESULTS

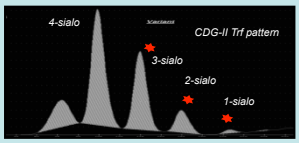
➢ Case 1: ATP6V0A2-CDG

Clinics



Unspecific multivisceral symptoms
Cutis laxa

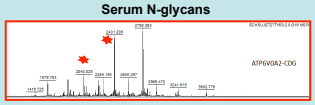
CDG screening
CE of transferrin (Trf)



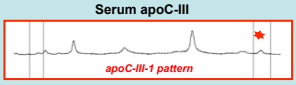
CDG-II Trf pattern suggestive of N-glycans maturation defects in the Golgi apparatus (GA).

MALDI-TOF MS

Serum N-glycans

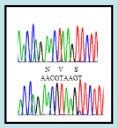


Serum apoC-III



-Hypo-sialylation/galactosylation of N-glycans..
-Hyposialylation of apoC-III.
→ Suspicion of ***ATP6V0A2 deficiency***

Gene sequencing



CDG diagnosis

ATP6V0A2-CDG

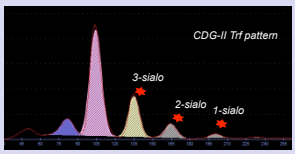
ATP6V0A2 is a protein involved in GA pH regulation

➢ Case 2: COG-CDG

Clinics

Unspecific multivisceral symptoms

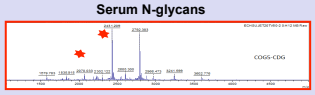
CDG screening
CE of transferrin (Trf)



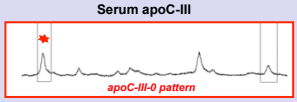
CDG-II Trf pattern suggestive of N-glycans maturation defects in the Golgi apparatus (GA).

MALDI-TOF MS

Serum N-glycans

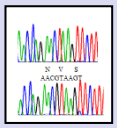


Serum apoC-III



-Hyposialylation/galactosylation of N-glycans.
-Hypoglycosylation of apoC-III.
→ Suspicion of ***COG deficiency***

Gene sequencing



CDG diagnosis

COG5-CDG

COG proteins complex acts as a tethering factor involved in GA retrograde trafficking

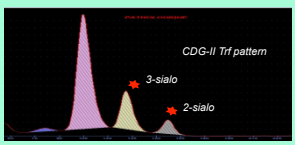
➢ Case 3: SLC35A3-CDG

Whole Exome sequencing

SLC35A3-CDG?
GlcNAc transporter deficiency ?

Diagnosis suspiscion

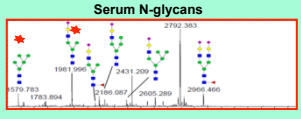
CDG screening
CE of transferrin



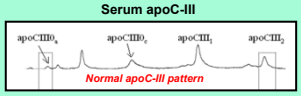
CDG-II Trf pattern suggestive of N-glycans maturation defects in the Golgi apparatus (GA).

MALDI-TOF MS

Serum N-glycans

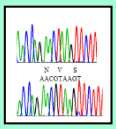


Serum apoC-III



- Multiple defects in N-GlcNAcylation.
- Normal apoC-III pattern
→ Strongly suggestive of ***SLC35A3-CDG***

Gene sequencing



CDG diagnosis

SLC35A3-CDG
GlcNAc transporter deficiency

Diagnosis confirmation

CONCLUSION

Whatever CDG could be suspected based on clinical symptoms (cases 1 & 2) or whole exome sequencing data (case 3), MALDI-TOF MS of serum N-glycans and of entire mucin core1 O-glycosylated apoC-III give important structural informations greatly orientating the diagnosis pathway towards accurate determination of causative gene mutations.