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SLC37A4-CDG: New biochemical insights for an emerging congenital disorder of glycosylation with major coagulopathy

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ABSTRACT

SLC37A4-CDG is an emerging congenital disorder of glycosylation which is characterized by a dominant inheritance and a major coagulopathy originating from the liver. Recent studies took interest in the biochemical alterations found in this CDG and showed that they consisted of multiple glycosylation abnormalities, which result from mislocalization of the endoplasmic reticulum glucose-6-phosphate transporter and associated Golgi homeostasis defects. In this work, we highlight in six affected individuals abnormal patterns for various serum N-glycoproteins and bikunin proteoglycan isoforms, together with specific alterations of the mass spectra of endoglycosidase H-released serum N-glycans. Collectively, these data complement previous findings, help to better delineate SLC37A4-CDG and could present interest in diagnosing this disease.

1. Introduction

Heterozygous SLC37A4 deficiency is a recently characterized congenital disorder of glycosylation (SLC37A4-CDG) with, to this day, nine described affected individuals sharing the c.1267C > T (p.R423X) variant [1–3]. In this dominantly inherited metabolic disease, the monoallelic loss of the retrieval motif of the endoplasmic reticulum SLC37A4 glucose-6-phosphate transporter leads to its partial mislocalization, with deleterious impacts on liver Golgi homeostasis (pH and morphology), glycosylation and coagulation factors levels [3]. All affected individuals showed altered N- and mucin-type O-glycosylation profiles, elevated ASAT and decreased F2, F11 and antithrombin (AT) levels. In this context of major coagulopathy, most patients nevertheless safely benefited from more or less invasive surgery including lip/palate cleft, tongue-tie and heavy cardiac surgeries, under fresh frozen plasma or not [3]. This suggests a preserved balance between pro-coagulant (e.g., F2 and F11) and anti-coagulant factors (e.g., AT), although this must be confirmed in additional patients. Furthermore, given its rather reduced symptomatology and dominant inheritance, SLC37A4-CDG may be widely underdiagnosed.

In this work on six SLC37A4-CDG affected individuals, we expand the previously reported biochemical features by analyzing other relevant serum glycoproteins, including bikunin proteoglycan isoforms, and by performing additional matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) studies of mannosylated and hybrid-type serum N-glycans.

Abbreviations: 2-DE, two-dimensional electrophoresis; A1AG, α1-acid glycoprotein; AT, antithrombin; ASAT, aspartate aminotransferase; Bkn, bikunin; CDG, congenital disorder(s) of glycosylation; CS, chondroitin sulfate; ECL, electrochemiluminescence; Endo H, endoglycosidase H; 'HC' protein, 'heavy chain' protein; Hpt, haptoglobin; ITI, inter-α-trypsin inhibitor; MALDI-TOF, matrix assisted laser desorption/ionization-time of flight; MS, mass spectrometry; MW, molecular weight; PαI, pro-α-trypsin inhibitor; Trf, transferrin.

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2. Materials and methods

2.1. Serum samples

Serum samples were obtained from six SLC37A4-CDG affected individuals (P1 to P6) previously reported by Ng et al. [3].

2.2. Western blot of serum N-glycoproteins and two-dimensional electrophoresis (2-DE) of haptoglobin

Western blots of three N-glycoproteins, transferrin (Trf), haptoglobin (Hpt) and α1-acid-glycoprotein (A1AG), were performed on the six samples. SDS-PAGE was performed (10 μL of treated sample per well) using Nu-PAGE 4–12% bis-tris gels (ThermoFisher Scientific). Proteins were transferred onto nitrocellulose, incubated with primary rabbit antibodies (anti-Trf from Siemens, anti-Hpt and anti-A1AG from Dako; 1:3000 v/v in TTBS-5% milk), then with HRP-linked secondary antibodies (GE Healthcare; 1:5000 v/v) and detected using ECL revelation. 2-DE of Hpt was performed as previously described [4].

2.3. MALDI-TOF MS of endoglycosidase H-released serum N-glycans

Profiles of mannosylated and hybrid-type serum N-glycans from P1 to P6 were obtained by MALDI-TOF MS following N-glycan cleavage by *endo*-β-N-acetylglucosaminidase (Endo H), glycan purification by solid-phase extraction and permethylation, as previously described [5]. Mass spectra were acquired on an UltrafleXtreme instrument (Bruker Daltonics) operating in the positive reflector ion mode using 2,5-dihydroxybenzoic acid as matrix.

2.4. Western blots of serum bikunin isoforms

Western blots of serum bikunin (Bkn) isoforms were performed on four controls (C1 to C4) and on the six SLC37A4-CDG affected individuals. For inter-α-trypsin inhibitor (ITI: bikunin-chondroitin sulfate esterified by two HC proteins) and pro-α-trypsin inhibitor (PαI: bikunin-chondroitin sulfate esterified by one HC protein), sera were diluted 1:250 v/v in water. For unesterified Bkn-chondroitin sulfate and free Bkn, sera were diluted 1:10 v/v. SDS-PAGE, protein transfer and immunorevelation were performed as described above, with rabbit anti-bikunin (CP6) antibodies (Merck-Millipore, cat. # ABT1346; 1:5000 v/v in TTBS-5% milk).

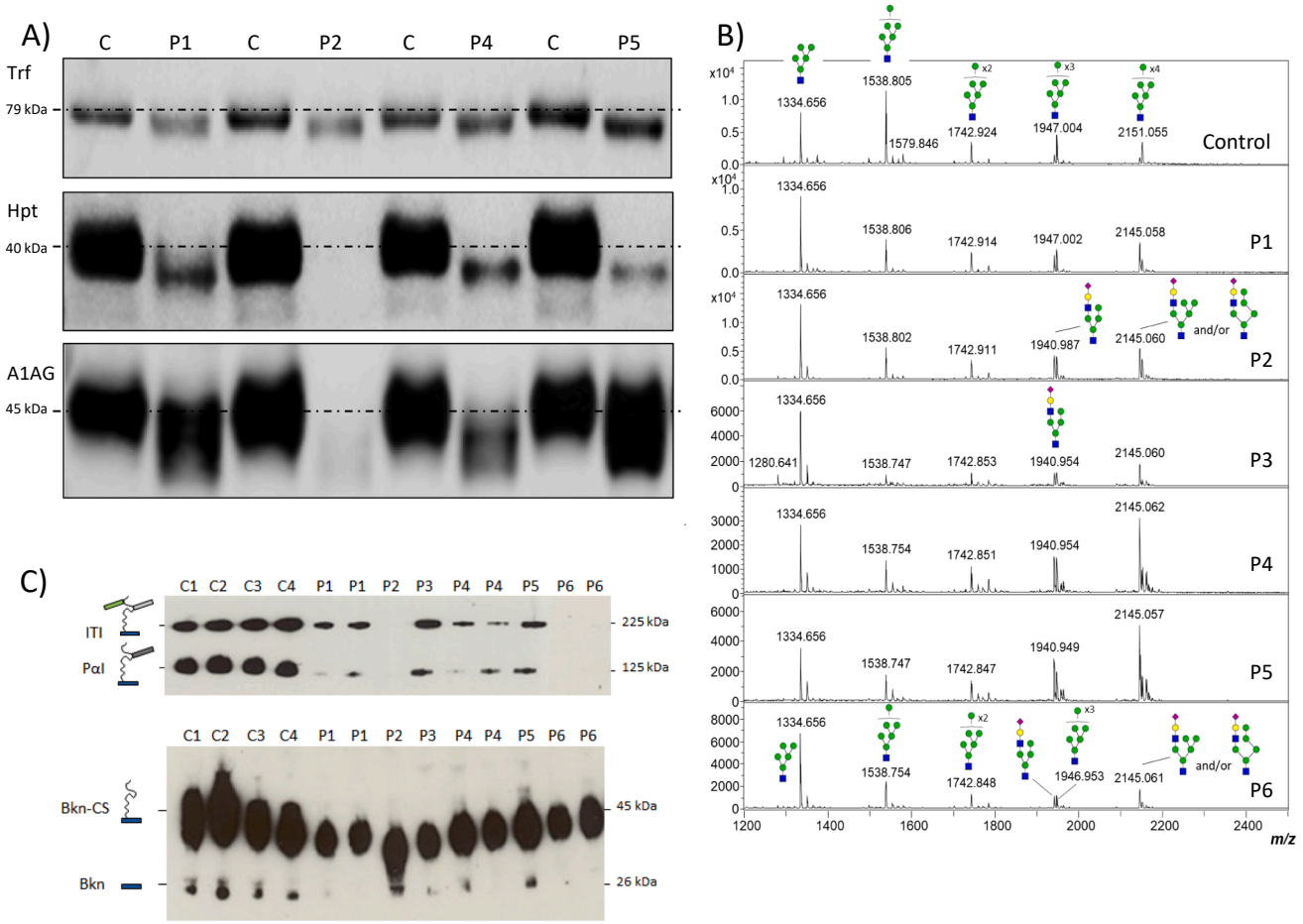

Fig. 1. Western blots of three serum N-glycoproteins, MALDI-TOF MS of Endo H-released serum N-glycans and western blots of bikunin isoforms, in SLC37A4-CDG. (A) Illustrative western blots of (from top to bottom) serum transferrin (Trf), haptoglobin (Hpt) and α1-acid-glycoprotein (A1AG) in four SLC37A4-CDG affected individuals (P1, P2, P4, P5) and a control (C). Dotted lines refer to normal glycoprotein MW. (B) MALDI-TOF mass spectra of permethylated N-glycans released from serum samples from a healthy subject (control) and six SLC37A4 patients (P1 to P6) following Endo H treatment. Measurements were performed in the positive-ion mode and all ions are present in sodiated form. Green circles, mannose; yellow circles, galactose; blue squares, N-acetyl glucosamine; red triangles, fucose; purple diamonds, sialic acid. (C) Western blots of serum bikunin (Bkn) isoforms in four controls (C1 to C4) and six SLC37A4-CDG affected individuals (P1 to P6). For P1, P4 and P6, two different samples were analyzed. ITI: bikunin (Bkn) linked to a chondroitin sulfate (CS) chain esterified by two HC glycoproteins; PαI: Bkn linked to a CS chain esterified by one HC glycoprotein. Profiles of controls were overexposed in order to be able to visualize ITI and PαI protein bands in some patients.

3. Results

3.1. Western blot of N-glycoproteins and 2-DE of Hpt

Representative western blots of Trf, Hpt and A1AG from P1, P2, P4 and P5 are presented in Fig. 1**A**. For all patients, glycoprotein bands clearly display molecular weight (MW) decreases compared to the control (C). In addition, 2-DE patterns of Hpt glycoforms (Supp. Fig. 1) showed that these MW decreases were combined to a loss of negative charges as they displayed an abnormal tray of protein spots of cathodical migration.

3.2. MALDI-TOF MS profiling of Endo H-released serum N-glycans

MALDI-TOF MS profiles of permethylated Endo H-released total serum N-glycans of the six patients and the control are shown in Fig. 1**B**. All patients shared a similar pattern, which notably displayed the abnormally prominent high-mannose $GlcNAcMan_5$ structure at *m/z* 1334.6. Besides, profiles also contained predominant hybrid mono-sialylated N-glycan species at *m/z* 1940.9 and *m/z* 2145.1.

3.3. Western blot of serum bikunin isoforms

Western blots of serum Bkn isoforms are shown in Fig. 1**C.** For both ITI and PαI, corresponding protein bands showed severely reduced levels in the affected individuals compared to the controls. For Bkn-chondroitin sulfate (Bkn-CS), patients' patterns were similar to those of the controls with the exception of a lower MW for P2.

4. Discussion

To further characterize the biochemical alterations in SLC37A4-CDG, we performed various experiments, consisting of western blots of serum N-glycoproteins and bikunin (Bkn) proteoglycan isoforms, and MALDI-TOF MS analysis of Endo H-released serum N-glycans. First, western blots of serum Trf, Hpt and A1AG showed systematic MW decreases, in line with a major N-glycosylation deficiency (Fig. 1**A**) as corroborated using 2-DE applied to Hpt (**Supp.** Fig. 1). Such altered western blot and 2-DE profiles are rather unusual since, in our experience, they are only retrieved in MGAT2-CDG [6] and B4GALT1-CDG [7], two CDG with very severe clinical phenotypes.

Furthermore, we previously showed that SLC37A4-CDG-related N-glycosylation defects consist of the accumulation of high-mannose and hybrid-type N-glycans, together with hyposialylated and hypogalactosylated structures [3]. To specifically highlight probable major mannosidase(s) defect(s), we performed complementary MALDI-TOF MS analysis of Endo H-released high mannose and hybrid-type serum N-glycans. All affected individuals shared a similar pattern (Fig. 1**B)**, which efficiently discriminated them from the healthy subject. Results were even more striking than after peptide-N-glycosidase F treatment [3] and were clearly different from those found in α1-2 mannosidase deficiency (MAN1B1-CDG), as reported elsewhere [5,8].

Lastly, SLC37A4-CDG was shown to be associated with an increased acidification of intraluminal Golgi pH [3]. Since we previously described altered ester linkages of the 'HC' proteins to the chondroitin sulfate (CS) moiety of the bikunin (Bkn) proteoglycan in CDG with Golgi V-ATPase proton pump disruption [9], we analyzed serum Bkn isoforms in the patients. Western blots of the heavy forms of Bkn (ITI and PαI) showed severely reduced levels compared to controls (Fig. 1**C**). Moreover, profiles of unesterified Bkn-CS were similar to controls with the exception of P2, who presented an abnormally low MW Bkn-CS isoform. In line with our previous work on Bkn isoforms [9], these patterns were consistent with a Golgi pH defect in all patients while P2 could possibly display an additional defect in the CS chain biosynthesis. Regarding the influence of pH, SLC37A4-CDG-associated Golgi pH decrease seems to have similar deleterious effects on the biosynthesis of Bkn heavy forms to that of a raised pH, as described in CDG with V-ATPase deficiencies [9]. However, the ITI/PαI ratio seems to be in favor of ITI in SLC37A4-CDG, in contrast with V-ATPase deficiencies where PαI was shown to be predominant [3]. Besides corroborating the high sensitivity of the Bkn heavy forms biosynthesis towards Golgi pH, these results suggest that the ITI/PαI ratio can distinguish between excessive decreasing or rising Golgi pH variations.

5. Conclusion

In summary, western blots of serum N-glycoproteins, MALDI-TOF MS analysis of Endo H-released N-glycans and western blots of bikunin isoforms highlighted new biochemical features of SLC37A4-CDG that are relevant to better delineate this newly described CDG while also providing seemingly characteristic patterns, with possible diagnostic implications.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.07.005.

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