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Regular Article

CCDC115-CDG: A new rare and misleading inherited cause of liver disease

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ABSTRACT

Congenital disorders of glycosylation (CDG) linked to defects in Golgi apparatus homeostasis constitute an increasing part of these rare inherited diseases. Among them, COG-CDG, ATP6V0A2-CDG, TMEM199-CDG and CCDC115-CDG have been shown to disturb Golgi vesicular trafficking and/or lumen pH acidification. Here, we report 3 new unrelated cases of CCDC115-CDG with emphasis on diagnosis difficulties related to strong phenotypic similarities with mitochondriopathies, Niemann-Pick disease C and Wilson Disease. Indeed, while two individuals clinically presented with early and severe liver fibrosis and cirrhosis associated with neurological symptoms, the other one “only” showed isolated and late severe liver involvement. Biological results were similar to previously described patients, including hypercholesterolemia, elevated alkaline phosphatases and defects in copper metabolism. CDG screening and glycosylation study finally led to the molecular diagnosis of CCDC115-CDG. Besides pointing to the importance of CDG screening in patients with unexplained and severe liver disease, these reports expand the clinical and molecular phenotypes of CCDC115-CDG. The hepatic involvement is particularly addressed. Furthermore, hypothesis concerning the pathogenesis of the liver disease and of major biological abnormalities are proposed.

1. Introduction

Congenital disorders of glycosylation (CDGs) are rare inherited diseases sharing very diverse and variable clinical symptoms related to defects in the glycosylation process of proteins and lipids. CDGs with abnormal protein N-glycosylation are classically sub-grouped as type I (CDG-I) or type II (CDG-II) according to affected metabolic steps. In CDG-I, the defect alters the oligosaccharide synthesis or its transfer to proteins in endoplasmic reticulum leading to N-glycosylation sites under-occupancy. In CDG-II, the defect alters the maturation of protein-linked oligosaccharide in the Golgi apparatus (GA) leading to the accumulation of N-glycan intermediate motifs [1]. In CDG-II subgroup, those related to disturbance of GA homeostasis constitute an increasing part with major involvement of deficient proteins normally involved in

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vesicular trafficking (COG-CDGs) and/or in pH regulation (ATP6V0A2-CDGs) [2]. Coiled-Coil Domain Containing 115 genetic deficiency (CCDC115-CDG) has been recently described in a single report of 8 patients sharing similar CDG-II transferrin profiles associated with hepatosplenomegaly (HSM), neurological symptoms, elevated serum aminotransferases (ATs) and alkaline phosphatases (ALP), hypercholesterolemia and Wilson disease (WD)-like disturbed copper metabolism [3]. Since CCDC115 protein is probably involved in the V-ATPase proton pump assembly, mutations have been suggested to alter GA lumen acidification, indirectly leading to glycosylation defects [3]. Furthermore, genetic deficiency in TMEM199, a CCDC115 interacting protein, has been concomitantly described presenting with milder neurological and hepatic symptoms and with very similar biological phenotypes [4]. The present work describes clinical, histological, biological, genetic, and glycosylation profiles of 3 newly identified unrelated cases of CCDC115-CDG. It expands the genotype (2 new mutations) and clinical phenotype of this disease and particularly points to multiple diagnosis difficulties related to clinical and biological symptoms suggesting either mitochondriopathy, Niemann Pick disease C (NPC) or Wilson disease (WD). In order to avoid diagnostic wavering, it also illustrates the importance of systematic CDG screening when confronted with unexplained and severe liver disease with or without associated neurological disorders.

2. Results

2.1. Clinical description

Patient1 (Pt1) is a French girl from non-consanguineous parents. She was born at term after an uneventful pregnancy. At birth, major hepatosplenomegaly (HSM) and cholestasis were reported. At 6 months of age, HSM was persistent with elevated liver enzymes but cholestasis spontaneously normalized. Psychomotor development was mildly delayed with hypotonia. At that time, based on neurological involvement and important HSM, NPC disease was suggested but biologically and genetically excluded. At 4 years of age, in a context of persistent and unexplained HSM and cytolysis, a needle liver biopsy was performed showing a very fragmented liver specimen, with several portal-to-portal bridging fibrosis without regenerative nodules, fatty hepatocytes, foamy histiocytes and iron overload. At 13 years of age, she had subnormal intellectual capacities but behavioral troubles with intolerance to frustration, aggressiveness and agitation. Brain magnetic resonance imaging (MRI) was normal. She showed mild dysmorphic features and persistent HSM. Aminotransferases (ASAT/ALAT) and alkaline phosphatases (ALP) were discreetly elevated (ASAT = 56 U/L, ALAT = 45 U/L, N < 40 U/L; ALP = 196 U/L, N < 119 U/L) while γ-glutamyltransferase (GGT), bilirubin and CK were normal. She had mild hypercholesterolemia (5.50 mmol/L, N < 5.20 mmol/L) and normal glucose and lactic acid. Metabolic investigations including plasma amino acids, urinary organic acids, and lysosomal acid lipase were regarded as normal. Chromatographic analysis of the lipids from the liver showed high level of Bis(Monoacylglycero)Phosphate (not shown). Copper metabolism-related findings were low serum ceruloplasmin (0.55 µmol/L, N = 2–4.5 µmol/L), low serum total copper (5.44 µmol/L, N = 12.5–22.5 µmol/L), slightly elevated relative exchangeable copper (REC = exchangeable copper/total copper %) (10.7%, N = 3.0–8.1%) and elevated urinary copper (0.48 to 1.89 µmol/24 h, N < 0.40 µmol/24 h). Kayser-Fleisher ring was absent and *ATP7B* gene sequencing failed to diagnose Wilson disease (WD). At 16 years of age, a second liver biopsy showed enlarged portal tracts with slender portal-to-portal bridging fibrosis (stage F2 in METAVIR scoring system) (Fig. 1A, B). A proliferation of bile ductules was present in some portal tracts with apoptotic bodies and nuclear dystrophy of cholangiocytes (Fig. 1C–D). Macro-and micro-steatosis was slight (< 5%) but focally present (Fig. 1E). Rhodanine copper staining showed faint cytoplasmic staining in isolated periportal hepatocytes (Fig. 1F) and also glycogen vacuolization in some hepatocyte nuclei. Signs of steatohepatitis such as Mallory–Denk bodies, ballooned hepatocytes and active portal inflammation, were absent.

Patient 2 (Pt2) is a French man from non-consanguineous parents. No specific medical history was reported until he was 20 years old. He was referred for chronic hepatitis with elevated serum liver enzymes i.e., ALAT = 154 U/L and ASAT = 137 U/L (N < 40 U/L) for > 6 months. Serum GGT level was normal (35 U/L; N < 40 U/L) but ALP was increased (218 U/L; N < 119 U/L). Serum CK were elevated (519 U/L; N < 190 U/L) without clinical muscular disease. He had marked hypercholesterolemia (8.16 mmol/L; N < 5.7 mmol/L) with an increase of LDL-cholesterol despite normal body mass index. No splenomegaly or hepatomegaly was found. Psychomotor development was normal without dysmorphic features. Ceruloplasmin concentration was low (0.54 µmol/L; N = 2–4.5 µmol/L) and the 24 h-urinary copper excretion was normal (0.20 µmol/24 h; N < 0.40 µmol/24 h). Moreover, REC was increased (19%; N = 3.0–8.1%) as well as liver copper concentration (2.47 µmol/g, N = 0.3–0.9 µmol/g). Kayser-Fleisher ring was absent and cerebral MRI was normal. Liver biopsy showed portal fibrosis with numerous portal-to-portal bridging fibrosis without cirrhosis (stage F2/F3 in METAVIR scoring system) and partial loss of interlobular ducts. A proliferation of bile ductules was present in some portal tracts. Taking into account the low ceruloplasmin concentration, the clinical profile was rather consistent with WD. D-penicillamin treatment (600 mg/day) was started while waiting for the results of *ATP7B* gene sequencing. Two months later, liver blood tests were not improved and urinary copper excretion was not increased. D-penicillamin therapy was stopped. None *ATP7B* gene mutation was found and WD diagnosis could not be asserted. MRI liver cholangiography was normal and autoimmune hepatitis or primary sclerosing cholangitis were ruled out as well as all other causes of acute and chronic liver disease.

Patient 3 (Pt3) is a French boy from non-consanguineous parents. He was born at term after an uneventful pregnancy. At birth, axial hypotonia, major HSM and cholestasis were reported. Explorations ruled out all classical causes of neonatal cholestasis [5] and also Gaucher and Niemann-Pick diseases. He had particular high serum cholesterol (9.92 mmol/L; N < 5.2 mmol/L), and acid lipase deficiency was also excluded. In absence of diagnosis, a liver biopsy was performed at 2 months of age, and identified enlarged portal tracts with several portal-to-portal bridging fibrosis (stage F3 in METAVIR scoring system), focal portal inflammation, loss of interlobular ducts in 1/3 of portal tracts and proliferation of bile ductules. A non-systematized microvacuolar steatosis was present (70%). There was no foamy hepatocyte or bile plug. A mitochondrial cytopathy was suspected in absence of other causes of liver disease despite normal lactate serum level. Enzymatic measurements in the liver identified isolated mitochondrial complex III (CIII) enzyme deficiency, which was not observed in lymphocytes and fibroblasts. Clinical evolution led to cirrhosis presentation with enlarged liver and portal hypertension. At this stage, a moderate delay in the global development was identified. A second liver biopsy was performed at 2.5 years of age and identified micronodular cirrhosis with non-inflammatory fibrosis, mild bile ductular proliferation. Lobular steatosis persisted with very mild intensity (10%). CIII liver deficiency was confirmed on this second sample. Mutation in the CIII gene and deletion in the mitochondrial DNA were discarded. In this context, sequencing of a panel of 215 genes involved in the mitochondrial metabolism was performed and did not identify any disease-causing mutation. CGH-Array was normal. Pt3 developed moderate mental retardation (normal brain MRI) and had also elevated CK and absent deep tendon reflex. For this reason, a muscular biopsy was performed at the age of 16 years. No ragged-red fibers were observed with Gomori Trichrome. The cytochrome oxidase was normal and the succinic deshydrogenase showed thin darkly cytoplasmic rim of mitochondria in a minority of muscle fibers, against the diagnosis of mitochondrial cytopathy. CIII of the muscular mitochondrial chain was normal. The PAS

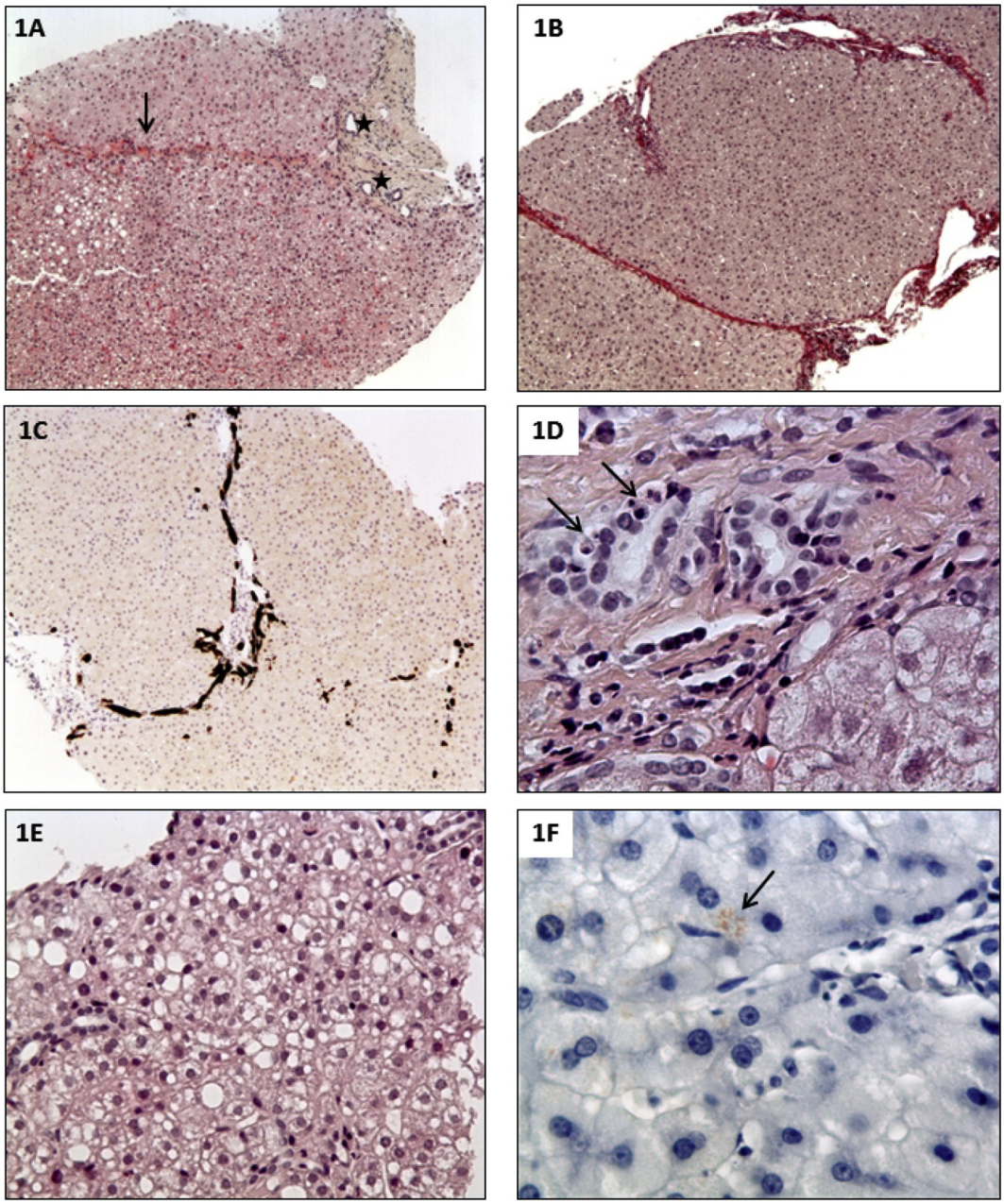

Fig. 1. 1A. Slender portal-to-portal bridging fibrosis (arrows). Irregular sized ductular proliferation (asterisks) (HES staining). 1B. Slender portal-to-portal bridging fibrosis, METAVIR F3 (Picro-Sirius staining). 1C. Proliferation of bile ductules highlighted with cytokeratin 7 staining. 1D. Apoptotic bodies, nuclear dystrophy of cholangiocytes (arrow). Note slight portal inflammation (HES staining). 1E. Focal macro-and micro-steatosis (HES staining). 1F. Faint cytoplasmic copper staining in isolated periportal hepatocytes (Rhodanine staining).

staining showed a normal glycogen content in the majority of muscle fibers, only scattered fibers were free of glycogen and contained alpha-amylase resistant periodic acid Schiff positive cytoplasmic inclusions. A type IV glycogen storage disease was suspected but ruled out after negative molecular analysis. Lastly, exome sequencing was performed and identified compound heterozygous mutation in *CCDC115* gene. Supplementary explorations at the age of 18 years confirmed persistent hypercholesterolemia and showed disturbed copper metabolism. Ceruloplasmin concentration was low (0.09 g/L; N = 0.2–0.5 g/L), REC was increased (18%; N = 3.0–8.1%) while serum copper (4.16 µmol/L) and exchangeable copper (0.75 µmol/L) were normal.

2.2. Glycosylation study

Mainly because of the unexplained liver involvements, a CDG screening and additional glycosylation studies were performed separately in both patients' sera and showed very similar glycosylation defects. Capillary electrophoresis of serum transferrin showed a CDG-II pattern (Fig. 2A) which was in agreement with the MALDI-TOF MS profiles of serum N-glycans, indicating essentially accumulation of (i), partially sialylated biantennary N-glycans (*m/z* 2605.3 and 2431.2) and of (ii), N-glycans lacking both sialic acid and galactose residues (*m/z* 2227.1 and 2040.0). Also, core fucosylation seemed slightly increased (*m/z* 2040.0, 2605.3 and 2966.5) (Fig. 2B). Two-dimensional electrophoresis and MALDI-TOF MS of mucin core1 O-glycosylated apolipoprotein C-III also showed O-glycans sialylation defects with a decrease

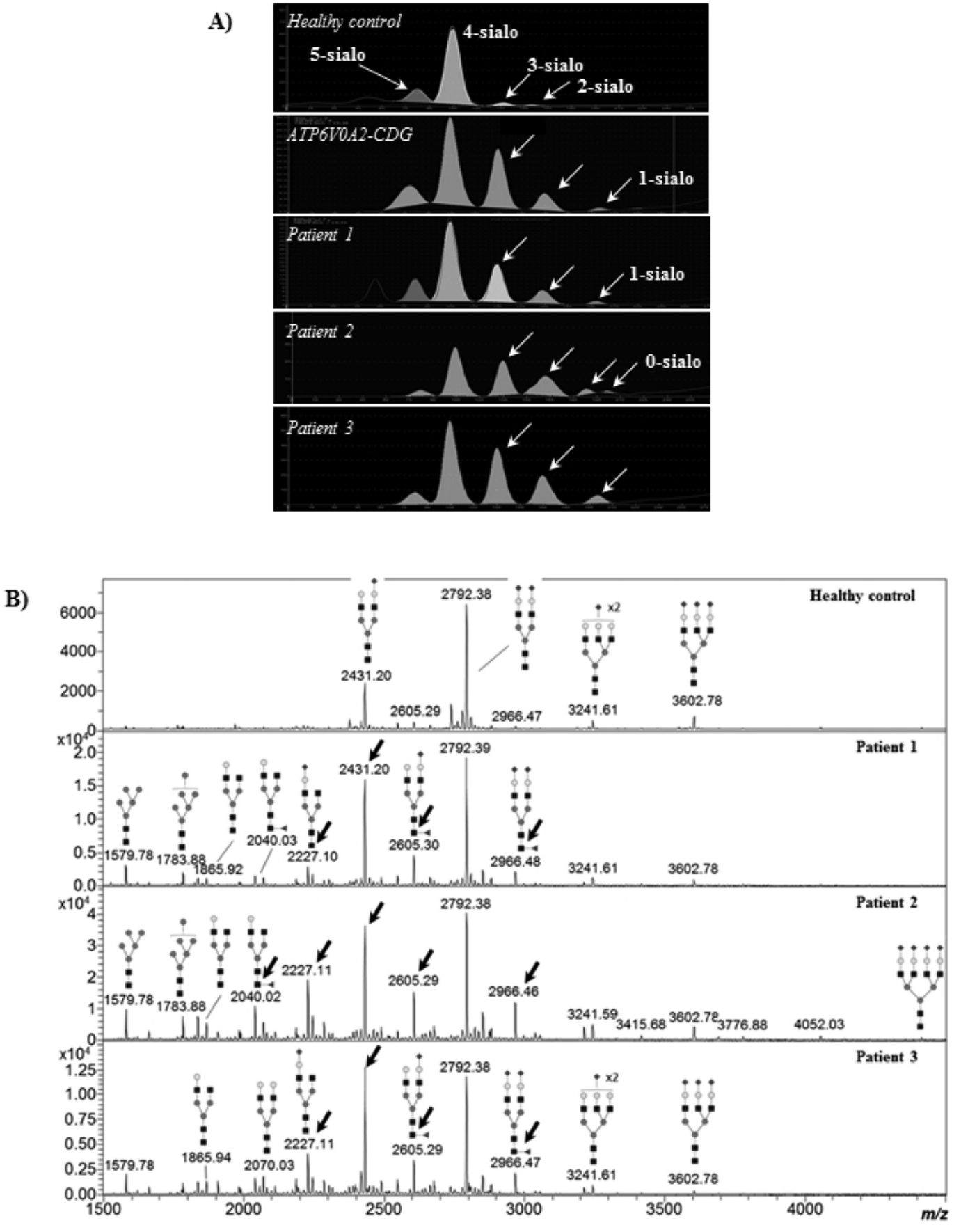

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Fig. 2. Glycosylation studies 2A. Transferrin glycoforms profiles: Serum transferrin capillary electrophoresis patterns of Pt1 and Pt2 (lower profiles) by comparison with control and ATP6V0A2-CDG (upper profiles). Similarly to ATP6V0A2-CDG, it can be observed in patients, typical CDG-II patterns showing decreased level of tetra-sialotransferrin (4-sialo) coupled to increased levels of hyposialylated glycoforms (0- to 3-sialo). 2B. MALDI-TOF MS of N-glycans: When compared with control (upper profile), MALDI-TOF MS of serum N-glycans of patients (lower profiles) showed relative accumulation (arrows) of partially sialylated biantennary N-glycans (m/z 2605.3 and 2431.2) and of N-glycans lacking both sialic acid and galactose residues (m/z 2227.1 and 2040.0). Also, core fucosylation seemed slightly increased (m/z 2040.0, 2605.3 and 2966.5).

of the bi-sialylated glycoform percentages (Supplementary files).

2.3. Molecular study

Based on presented phenotypes associated to typical N- and O-glycosylation abnormalities, CCDC115 and TMEM199 encoding gene were sequenced in the 3 individuals. For Pt1, two mutations were found at heterozygous state in exon 1 of *CCDC115* gene: one missense mutation c.92T > C p.Leu31Ser (rs751325113), already described in CCDC115-CDG patients [3], and a new nonsense mutation c.19C > T p.Arg7* (rs374624586). Pt2 had two missense mutations in exon 1 of *CCDC115* gene at heterozygous state, the already described c.92T > C p.Leu31Ser and a new mutation c.38T > C p.Leu13Pro predicted as being pathogenic by Polyphen2 software package. Pt3 shared exactly the same mutations than Pt1. All mutations were found < 0.01% in ExAc database and were inherited each from one parent. Taken together, these results asserted CCDC115-CDG inherited in an autosomal recessive manner for the 3 patients. All mutations identified in this study concerned the first exon encoding for the first predicted coiled-coil domain of the protein. Lastly, no mutations were found in TMEM199 encoding gene for the 3 patients (not shown).

3. Discussion

CCDC115-CDG was recently discovered by Jansen et al. reporting 8 affected individuals from 5 families [3]. Besides CDG related N- and O-glycans abnormalities, key features of this new disease were described as psychomotor disability (PMD) and marked HSM associated with elevated ATs and ALP and hypercholesterolemia. Furthermore, basic copper metabolism parameters were assessed in 5/8 patients showing decreased serum ceruloplasmin and copper. We described here 3 additional unrelated CCDC115-CDG cases. Because of multiple clinical overlaps with various other inherited liver diseases, screening and definitive diagnosis were difficult and sometimes (except for Pt2) greatly delayed. Indeed, Pt1 presented at birth with neonatal cholestasis, hypotonia and major HSM. When associated to marked neurological symptoms and lipids abnormalities, the main diagnosis hypothesis was NPC disease. But it was biochemically (normal Filipin test) and genetically ruled out and no other diseases, including WD, could be asserted until CDG was identified at 16 years old. Pt2 presented at 20 years old without any medical history and showing isolated elevated ATs. Despite the absence of neurological symptoms and atypical lipid and CK abnormalities, WD was suspected based mainly on hypoceruloplasminemia. But WD could not be asserted (no identified ATP7B mutations, D-penicillamin inefficiency, normal 24 h-urinary copper excretion) until CDG diagnosis at 22 years old. Pt3 presented at birth with severe cholestasis, HSM and axial hypotonia, which first suggested NPC disease. Since the latter was genetically excluded, liver and neurological phenotypes, when associated to liver CIII deficiency, secondly evoked possible mitochondriopathy. But corresponding genetic study i.e., the sequencing of a panel of 215 mitochondria-related genes was unsuccessful. With time, associated moderate mental retardation and mild muscular involvement led to suspect atypical type IV glycogenesis that was also discarded. Finally, the diagnosis of CDG was made at 17 years old.

The neurological phenotype of these 3 new patients, ranging from absence of symptoms (Pt2) to PMD and hypotonia (Pt1 and Pt3), expanded the clinical spectrum of CCDC115-CDG, overlapping it with TMEM199-CDG (showing mild neurological symptoms) [4]. The liver phenotype appeared to be rather homogeneous associating severe fibrosis for all patients with HSM for 2 among 3. Histologically, we observed in all cases portal-to-portal bridging fibrosis with cirrhosis in one case. At the beginning of the disease, some portal inflammation, some decrease of the number of interlobular ducts and a bile ductular proliferation were present in all cases. Central veins and microcirculation were normal and steatosis was present in all cases, mainly focal (5 to 10%). Copper rhodanine staining showed slight hepatocyte accumulation in periportal area for one case. Mild elevated liver-copper concentration was observed in another case. Despite clinical signs of cirrhosis for Pt1 and Pt3, they did not develop signs of severe portal hypertension with over 15 years of follow-up. Furthermore, Pt1 had a liver biopsy at 16 years old, which did not evidence major fibrosis progression. By contrast, 3 among the first patients described by Jansen et al. [3] developed severe liver failure indicating that hepatic involvement could also be variable in CCDC115-CDG. Concerning biological data, we retrieved in all patients: hypercholesterolemia, elevated ATs and PAL and hypoceruloplasminemia, as previously described. Together with typical N- and O-glycosylation defects (i.e. hypo-galactosylation/sialylation of N-glycans and hyposialylation of apoC-III), these biochemical abnormalities appeared to be highly conserved among all CCDC115-CDG patients. The respective involvements of liver cirrhosis and CDG in the genesis of the majority of these biological defects could be questioned and will need deeper evaluation. Lastly, elevated CK observed in some patients, could suggest possible associated muscular involvement.

CCDC115 protein has been shown to be mainly located in the ER-Golgi intermediate compartment (ERGIC) in Hela cells and immortalized human hepatocytes and was proposed to participate (with TMEM199) in Golgi vacuolar type H^+ ATPase (V-ATPase) proton pump assembly [3]. Similar roles at the lysosomal-endosomal system have also been described, in agreement with CCDC115 localization in mouse [6]. Furthermore, whether this protein is implied in V-ATPase-related trafficking and/or pH regulation functions needs additional investigations.

The main assumption regarding the pathogenesis of hepatopathies (i.e. steatosis and fibrosis leading to cirrhosis) in CCDC115-CDG patients is a defect in the liver autophagy process. Indeed, in the field of CDG, it has been recently reported that genetic deficiency in ATP6AP2 protein, another subunit of the V-ATPase complex, was notably associated with very similar liver phenotypes coupled to marked autophagic dysregulation [7]. Since this latter was shown to be tightly associated with defects in lysosomes acidification and mTor signaling, a similar process could also be highly suspected in CCDC115 deficiency. Furthermore, defective autophagy has been described as promoting steatosis in non-alcoholic fatty liver disease [8] and under high fat diet [9]. Also, a few data suggested that defects in autophagy could contribute to liver fibrosis (in balance with the inhibition of the “activation” process of hepatic stellate cells) by triggering apoptosis of hepatocytes and by reducing anti-inflammatory properties of macrophages [10,11]. In this context, it should be interesting to assess the liver autophagic status of misleading NPC, WD and mitochondriopathies by comparison with CCDC115-CDG and other related diseases including TMEM199-CDG.

Concerning glycosylation and other main biological abnormalities, we propose (as schematized in Fig. 3) that CCDC115 mutations globally affect GA and lysosomal-endosomal homeostasis leading to defects in N- and O-glycosylation as well as in cholesterol and copper intracellular

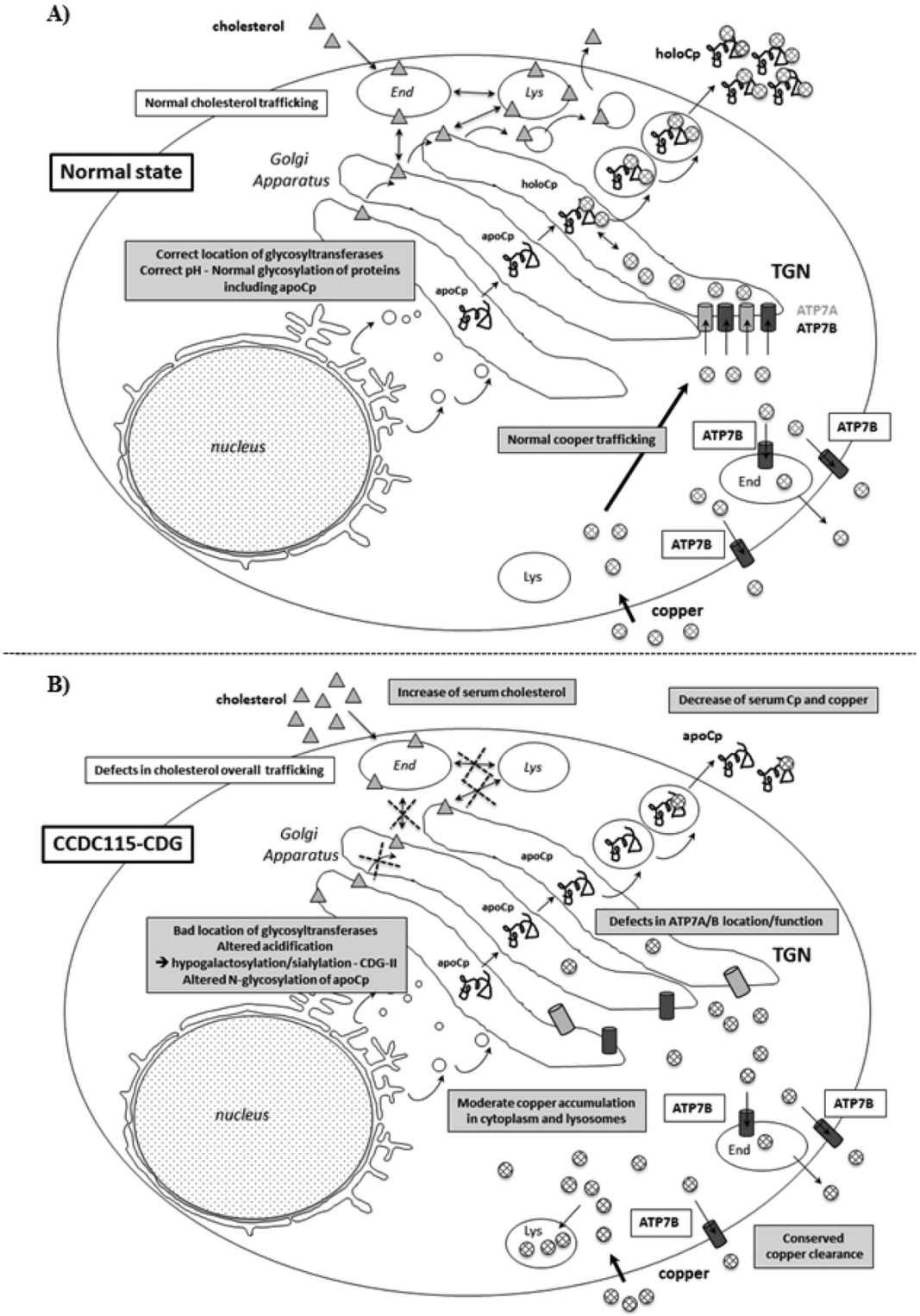

Fig. 3. Suggestion of an integrative view of CCDC115-CDG related GA defects affecting glycosylation of proteins, cholesterol trafficking (triangles) and copper circulation (grid circles) into hepatocytes. A: in normal state; B: in CCDC115-CDG. "Lys" = lysosomes; "End" = endosomes; "TGN" = trans-Golgi Network; "apoCp" = apoceruloplasmin; "HoloCp" = holoceruloplasmin.

trafficking. More precisely, as described in ATP6V0A2-CDG [12], CCDC115-CDG associated glycosylation defects could be related to disturbed Golgi trafficking and/or acidification impacting location and function of glycosyltransferases and leading to N- and O-glycans abnormalities (hypo-galactosylation/sialylation of N-glycans and disturbed sialylation of mucin core1 O-glycans). Concerning hypercholesterolemia, although a Golgi trafficking defect could also be evoked, it has been shown that disturbed endosomal/lysosomal acidification indirectly impairs normal trafficking of cholesterol [13], which could lead to its higher serum concentration in CCDC115-CDG. Furthermore, since Bis(Monoacylglycero)-Phosphate membranous level has been shown to greatly impact lysosomal cholesterol homeostasis in

NPC [14], its observed increase in the liver of Pt1 (not measured in other patients), appears as a possible interesting clue for the better understanding of CCDC115-CDG related cholesterol metabolism defects.

Concerning copper, once imported in the cytoplasm of hepatocytes, it normally enters the trans Golgi network (TGN) thanks to ATP7A and ATP7B, two membranous transporters with ATPase activity. In the TGN, copper associates with the glycoprotein "apoceruloplasmin" forming "holoceruloplasmin", which is then delivered into the blood. Additionally, in response to rising copper levels in the cytosol, copper can associate with ATP7B redistributed to plasma membrane or into endosomal-like vesicles, before being secreted/excreted [15] (Fig. 3A). In CCDC115-CDG (Fig. 3B), disturbed GA trafficking probably induced bad location of ATP7B and ATP7A as well as altered N-glycosylation of apoceruloplasmin. Furthermore, ATPase enzymatic activities of ATP7A/B could be deficient due to impaired TGN and endosomal acidification. Under these pathological conditions, copper bile excretion could be disturbed leading to its tendency to accumulate in cytosol and lysosomes of hepatocytes. Nevertheless, since liver copper accumulation appeared relatively moderate in our patients (as determined by liver histology or direct measurement), it can be speculated that ATP7B relocation at the plasma membrane is probably poorly affected allowing effective copper cellular clearance.

4. Conclusions

As corroborated in this work, genetic deficiencies in CCDC115 protein globally affect GA homeostasis impacting both N-glycosylation, O-glycosylation, intracellular cholesterol trafficking and copper metabolism. These "Golgilation" defects seem to be systematically associated with severe liver involvement in a misleading ambiance of other inherited liver diseases such as NPC disease, Wilson disease and mitochondriopathies. Thus, we strongly recommend CDG screening in all patients with unexplained liver disease associated with hypercholesterolemia and disturbed copper metabolism.

5. Materials and methods

5.1. Capillary electrophoresis of transferrin

Separation of serum transferrin glycoforms was carried out as previously described [16] using capillary zone electrophoresis method (Sebia Capillarys® CDT).

5.2. Mass spectrometry-based profiling of serum N-glycans

Sample processing for N-glycomic profiling of the serum samples was carried out essentially as described previously [17]. The serum samples (5 µL) were diluted in 20 mM sodium phosphate buffer (pH 7.4) and 10 mM dithiothreitol solutions, and then heated at 95 °C for 5 min. Peptide N-glycosidase F digestion of the resulting solutions was then performed overnight at 37 °C (Roche Diagnostics, Meylan, France). After sample acidification, proteins were precipitated using ice-cold ethanol. Released N-glycans were purified using porous graphitic carbon solid phase extraction cartridges (Thermo Scientific, les Ulis, France). The native N- glycans were subsequently permethylated and purified on a C18 spin-column (Thermo Scientific) before analysis by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

The dried permethylated samples were resuspended in 10 µL of a 50% methanol solution. 0.5 µL of the sample was then spotted on the MALDI target and thoroughly mixed with 0.5 µL of 2,5-dihydroxybenzoic acid solution (10 mg/mL in 50% methanol containing 10 mM sodium acetate). Glycan analyses were performed on an UltrafleXtreme instrument (Bruker Daltonics, Bremen, Germany) operating in the reflectron positive ion mode. Manual assignment of glycan sequences was done from MS and MS/MS data on the basis of previously identified structures [16] and with the help of GlycoWorkBench software [18].

5.3. Two-dimensional electrophoresis and MALDI-TOF MS of apolipoprotein C-III

Two-dimensional electrophoresis and MALDI-TOF MS of mucin Core1 O-glycosylated apolipoprotein C-III were conducted as previously described [19].

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A competing interest statement

No competing interest.

Patient consent statement

Informed consent was obtained for the 3 presented patients.

Details of ethics approval

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (Uniform Requirements for manuscripts submitted to Biomedical journals).

Details of the contributions of individual authors

MG: clinical evaluation of Pt1; wrote the article with AB.
AP: clinical evaluation of Pt2; specialized in WD.
MF: significant participation in the design of the study, histology collection, drafting and critical revision of the article.
FL: clinical evaluation of Pt1.
DD: clinical evaluation of Pt3.
MR: genetics of Pt3
FF: mass spectrometry of N-glycans; supervision of mass spectrometry studies.
SC: mass spectrometry of N-glycans.
CR: mass spectrometry of apoC-III.
EC: biological evaluation of Pt2.
JS: liver histology of Pt2.
LBN: clinical evaluation of Pt1.
FW: clinical evaluation of Pt2; specialized in WD.
TD: CDG screening.
SVB: genes sequencing.
NS: CDG screening; supervision of the work related to glycosylation.
LA: clinical evaluation of Pt2.
PDL: clinical evaluation of Pt1; supervision of the work related to clinic.
AB: CDG screening; wrote the article with MG. supervision of the work related to glycosylation. And a special word of thanks to Antoine Legendre for his precious help in the elaboration of Fig. 3.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2018.05.002.

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