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RESEARCH REPORT

An Unexplained Congenital Disorder of Glycosylation-II in a Child with Neurohepatic Involvement, Hypercholesterolemia and Hypoceruloplasminemia

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Abstract We report on a 12-year-old adopted boy with psychomotor disability, absence seizures, and normal brain MRI. He showed increased (but initially, at 5 months, normal) serum cholesterol, increased alkaline phosphatases, transiently increased transaminases and hypoceruloplasminemia with normal serum and urinary copper. Blood levels of immunoglobulins, haptoglobin, antithrombin, and factor XI were normal. A type 2 serum transferrin isoelectrofocusing and hypoglycosylation of apoCIII pointed to a combined N- and O-glycosylation defect. Neither CDG panel analysis with 79 CDG-related genes, nor whole exome sequencing revealed the cause of this CDG. Whole genome sequencing was not performed since the biological parents of this adopted child were not available.

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Abbreviations

ALP	Alkaline phosphatase
apoCIII	Apolipoprotein CIII
AST, ALT	Serum transaminases
CDG	Congenital disorders of glycosylation
CK	Creatine kinase
GGT	Gamma glutamyltransferase
IEF	Isoelectrofocusing
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
Tf	Serum transferrin

Introduction

Congenital disorders of glycosylation (CDG) are due to defects in the glycoprotein and glycolipid glycan synthesis and attachment. Glycoprotein glycosylation defects comprise disorders of N-glycosylation, O-glycosylation, and combined N- and O-glycosylation disorders (Jaeken and Morava 2016; Wolfe and Krasnewich 2013). Defective N-glycosylation is usually diagnosed by finding an abnormal serum transferrin (Tf) isoelectrofocusing (IEF) pattern (Jaeken et al. 1984). A type 1 pattern points to a defect in glycan assembly (CDG-I; cytosolic or ER defect), and a type 2 pattern to a defect in glycan remodelling (CDG-II; Golgi defect). The diagnosis of some (mucin type 1)

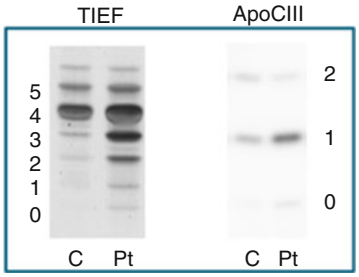

Fig. 1 Transferrin isofocusing for analysis of protein N-glycosylation showed increased disialo- and trisialotransferrin; isofocusing of apoCIII for analysis of mucin type O-glycosylation showed increased apoCIII-1 and decreased ApoCIII-2

O-glycosylation defects can be made by IEF of serum apolipoprotein CIII (apoCIII), showing a cathodal shift (Wopereis et al. 2003). The next step in the diagnosis, after excluding a protein variant and secondary hypoglycosylation, is mutation analysis of a panel of glycosylation genes. If this analysis reveals a normal result, whole exome sequencing should be performed and, if necessary, whole genome sequencing (Matthijs et al. 2013).

We report on a patient with a neurological presentation, transient biochemical liver involvement and other serum abnormalities, particularly hypercholesterolemia and hypoceruloplasminemia. Further investigation showed a type 2 serum Tf IEF and hyposialylation of apoCIII (Fig. 1). Neither CDG panel mutation analysis with 79 genes involved in CDG nor whole exome sequencing could reveal the cause of this CDG-IIx. Whole genome sequencing was not performed since the biological parents of this adopted patient were not available.

Case Report

This 12-year-old boy was adopted at the age of 8 months because his mother had intellectual disability. His birth weight was 3.500 g. He came to our attention at 5 months (weight 6.500 g, between 10 and 25th percentiles; length 67 cm, 50th percentile; head circumference 42 cm, 25th percentile) because of increased serum transaminases (AST: 100 U/L [normal: <41], ALT: 97 U/L [normal: <41]) and gamma glutamyltransferase (GGT) (286 U/L [normal: <71 IU/L]). Serum bilirubin, INR, aPTT, albumin, total and LDL cholesterol, and triglycerides were normal. Serum biliary acids were mildly increased (15.3 μmol/L; normal range 0–10). Alkaline phosphatase (ALP) levels were highly increased: 5,613 U/L (normal range: 300–850 U/L). Serum α1-antitrypsin (serum dosage, immunoelectrophoresis, and genetic test), autoantibodies (ANA, ASMA, LKM, AMA), celiac disease antibody tests, plasma amino acids, and urinary organic acids were normal.

He showed right plagiocephaly without facial asymmetry or dysmorphism. Neurological examination was normal and there was no hepatosplenomegaly. Tests for hepatitis B and C were negative and ultrasound abdominal examination was also normal. Serum Tf IEF showed a normal profile.

At 1 year, neurological examination was still normal. Serum transaminases had nearly normalized (AST 61, ALT 31 UI/L). GGT was normal as was creatine kinase (CK), but alkaline phosphatase was still high (1,574 U/L). Alkaline phosphatase isoenzyme analysis showed a 14% hepatic component (normal range 1–31%), a 76% bone component (normal range 62–100%), and a 9% biliary component (normal range 1–7%). Serum 25-OH vitamin D and 1,25-OH vitamin D were normal. His psychomotor development was relatively slow: he spoke his first words at 15 months and walked without support at 18 months.

At 22 months, a re-evaluation showed stable liver values and ALP profile, but a significant increase in cholesterol (total: 332 mg/dL, LDL-C: 240 mg/dL; HDL-C: 69 mg/dL) with persistently normal triglycerides (64 mg/dL), a normal apolipoprotein profile, INR, aPTT, antithrombin, factor XI. Serum copper was normal but ceruloplasmin was decreased (9 mg/dL [normal range: 20–40]). Twenty-four hour cupruria was repeatedly normal (<40 μg/24 h) and ATP7B mutation analysis was negative. Repeat IEF of serum Tf then showed a type 2 profile (increases in trisialo-, disialo-, monosialo-, and asialoTf) (Fig. 1). A Tf protein variant was excluded after neuraminidase treatment. Serum Tf glycan analysis using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) showed hyposialylation and mild hypogalactosylation (increase of a monosialo biantennary glycan and an abnormal peak corresponding to a monosialo-, monogalacto-biantennary glycan), (not shown). Also IEF of serum ApoCIII) showed hyposialylation (increased apoCIII-1 and decreased ApoCIII-2) (Fig. 1). Neither CDG panel mutation analysis with 79 genes involved in CDG nor whole exome sequencing revealed pathogenic variants. Whole genome sequencing was not performed since the biological parents of this child were not available.

His further evaluation showed mild developmental and speech disability. Brain MRI at 3½ and 8 years was normal. Physical examination, including liver and spleen, was normal. He attended school with a support teacher. At 8 years, he had repeated episodes of absence seizures with a pathological EEG that responded to levetiracetam.

He showed a progressive normalization of serum transaminases and GGT (last examination at 12 years: AST 33 U/L, ALT 15 U/L, GGT 10 IU/L), but persistently high ALP (957 U/L) and cholesterol levels (at 6 years: total cholesterol 351 mg/dL, LDL cholesterol 264 dL, HDL cholesterol 68 mg/dL).

Targeted resequencing of candidate genes (i.e. *LDLR, APOB, PCSK9, LDLRAP1, ABCG5/8*) was carried out by a NGS customized panel using the Ion PGM Sequencer. No variants with potential pathogenic effects were detected in the above-mentioned candidate genes. Also, in the WES data, these genes were looked at but no mutations found.

Serum ceruloplasmin remained low (7 mg/dL) with normal cupruria (<40 μg/24 h). Serum 25-OH vitamin D (5.3 ng/mL; normal range: 9–37) became subnormal despite oral supplementation. Serum levels of free T4, TSH, haptoglobin, IgG, IgA, and IgM were normal. Cholestyramine treatment, started at 8 years, led to a significant decrease in cholesterol levels (at 12 years: total cholesterol 220, LDL cholesterol 164, HDL cholesterol 42 mg/dL). A recent fibroscan showed no liver fibrosis.

Discussion

The patient discussed herein shows neurological involvement (learning difficulties and epilepsy) as well as biochemical evidence of a liver disorder. This is associated with a combined deficiency of N- and O-glycosylation, as shown by the type 2 pattern of serum Tf IEF and the hypoglycosylation pattern of serum apoCIII. Known CDG with a combined N- and O-glycosylation deficiency was excluded by CDG panel analysis and whole exome sequencing. A particular feature of this patient was his hypercholesterolemia. Since his biological parents could not be investigated, isolated familial hypercholesterolemia could not be excluded. The fact that at 5 months serum cholesterol was still normal may be in favor of an association with his CDG. Moreover, targeted resequencing of candidate genes of autosomal dominant and recessive hypercholesterolemias did not reveal either known pathogenic variants or novel variants of uncertain significance. Hypocholesterolemia is a well-known feature of PMM2-CDG and other CDG-I (Stibler et al. 1991; Weinstein et al. 2005; Tegtmeyer et al. 2014). It is usually attributed to a decrease in the cholesterol binding proteins apo A and apo B. Hypercholesterolemia is an exceptional feature in CDG. It has recently been reported in CCDC115-CDG (Jansen et al. 2016a) and TMEM199-CDG (Calvo et al. 2008; Jansen et al. 2016b), combined N- and O-glycosylation disorders of Golgi homeostasis. No explanation was provided for the hypercholesterolemia in these patients. Both these disorders, as well as the recently reported ATP6AP1-CDG (Jansen et al. 2016c), also showed hypoceruloplasminemia as does our patient. However, these patients also had hypocupremia and some had a mild increase in hepatic liver copper, differently from our patient. Hypoceruloplasminemia is not a recent finding in CDG; in fact, it has been reported in CDG-I, particularly PMM2-CDG, as one of the many glycoprotein abnormalities characteristic of these CDG (Harrison and Miller 1992; Henri et al. 1997; Heywood et al. 2016). However, the other investigated glycoproteins were normal in our patient. Mandato et al. (2006) also reported on four patients with subclinical liver involvement, a type 2 serum Tf IEF, and hypoceruloplasminemia. Differently from our patient, they did not have any neurological involvement, patients 1 and 2 also showed decreased serum copper and patients 3 and 4 a persistent decrease of multiple coagulation factors. The CDG type(s) in their patients and in our patient remain(s) to be determined.

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Take Home Message

Hypercholesterolemia and hypoceruloplasminemia, in a child with neurohepatic involvement, could be due to a congenital disorder of glycosylation, sometimes, as in this case, still unexplained.

Pier Luigi Calvo, Jaak Jaeken conceptualized and designed the study and drafted the initial manuscript.

Marco Spada, Ivana Rabbone, Michele Pinon, Francesco Porta, Fabio Cisarò, Stefania Reggiani, Angelo B. Cefalù, L. Sturiale, D. Garozzo, Dirk J. Lefeber conceptualized and designed the study and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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