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Sweet ending: When genetics prevent a dramatic CDG diagnostic mistake

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ABSTRACT

Herein, we described the case of a newborn male, from consanguineous parents, who developed, at day 11 of life, an obstructive hydrocephalus resulting from bilateral cerebellar hemorrhage without evident cause. Then, at 1 month, he developed a fulminant hepatitis with hyperammonia, hyperlactatemia and metabolic acidosis. Infectious and first line metabolic explorations were normal. Screening for congenital disorder of glycosylation (CDG) was performed using capillary electrophoresis and western blot of serum transferrin. Abnormal results were evocative of mannose-phosphate isomerase deficiency (MPI-CDG or CDG-Ib) as it can be responsible for fulminant hepatitis, digestive disease, developmental delay, and coagulopathy. However, trio whole exome sequencing revealed a pathogenic variant at the homozygous state in *ALDOB*, responsible for hereditary fructose intolerance (HFI), an inherited metabolic disorder with excellent prognosis under a fructose-free diet. HFI had not been previously evoked in view of the absence of diet diversification, but meticulous inquiry revealed that parents systematically added white sugar to the bottle milk of their child, unintentionally triggering potentially fatal HFI decompensations. Early genetic analysis upsetted both diagnosis and prognosis for this infant who had excellent development after fructose removal. This full-of-surprises diagnostic approach illustrates the importance of an integrative collaboration between clinicians, biochemists, and geneticists.

1. Case report

We report the case of a newborn male, first from consanguineous Afghan parents, without specific familial history. He was born after a full-term and uneventful pregnancy with normal birth measurements. Spatula extraction was needed, and he experienced a neonatal transient respiratory distress with rapid recovery and large serosanguinous hump.

Discharged from maternity at day 3 of life with mixed feeding (human milk and formula), he was readmitted at day 11 for vomiting, transient jaundice and hypotonia. Brain scan revealed bilateral cerebellar hemorrhage and multiple subarachnoid infra- and supratentorial hemorrhages responsible for an obstructive hydrocephalus. External ventricular drain was performed with favorable development. Etiologic investigations were inconclusive with normal ocular fundus and absence of arteriovenous malformation on brain MRI (Magnetic Resonance Imaging). Nevertheless, several hemostasis parameters were disturbed: prothrombin time (PTT) 57 % (normal range (NR) > 65 %), Factor II 57 % (NR > 60 %), Factor V 90 % (NR > 55 %), Factor XI 17 % (NR > 50 %), Factor XIII 27 % (NR > 60 %), and fibrinogen 0.84 g/L (NR: 1.6–4 g/L), but insufficiently to explain spontaneous hemorrhages in the opinion of hemostasis specialist. In the end, hemorrhage was allocated to the traumatic instrumental birth, and he was discharged.

At 1 month of life, he was referred again to emergency unit for hypotonia, drowsiness and vomiting, suggestive of hydrocephalus

Abbreviations: CDG, congenital disorder of glycosylation; CE, capillary electrophoresis; HFI, hereditary fructose intolerance; PTT, prothrombin time; Trf, transferrin.

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recurrence. Neurological investigations were normal, but biochemistry exploration revealed fulminant hepatitis without cholestasis: aspartate transaminases (AST): 4199 UI/L (NR < 40), alanine transaminases (ALT): 2991 UI/L (NR < 41), normal bilirubin and gamma-GT, PTT < 10 %, Factor V < 5 %, hyperammonia (179 μmol/L; NR < 60), metabolic acidosis (pH 7,06) with increased anion gap and hyperlactatemia (10.1 mmol/L; NR < 2). Transferred to the intensive care unit, he was treated by exclusive carbohydrate infusion, ammonia scavengers, vitamin therapy, large-spectrum antibiotics, and antiviral therapy. Despite a very worrying initial clinical presentation, all parameters quickly improved in a few days.

Large-scale infectious investigations were normal (hemoculture, cytobacterial examination of the urine, cytomegalovirus, Epstein-Barr virus, hepatitis A, B, C and E virus, herpes human virus 6, herpes simplex 1 and 2 virus, enterovirus, parvovirus B19, varicella-zoster virus, and toxoplasmosis were negatives), as were first line metabolic explorations, i.e., blood amino acids and acylcarnitines profile, urinary organic acids profile, ferritin, and redox balance. Tyrosinemia and galactosemia were also biochemically excluded. Abdominorenal ultrasound did not reveal hepatomegaly or portal hypertension signs but highlighted an unexplained bilateral nephrocalcinosis without noticed phosphocalcic alteration. Cardiac ultrasound was normal. The patient developed large thrombus on the central catheter after 5 days of management. Furthermore, large-scale hemostasis investigations revealed multiple abnormalities including decreased Factor XI (33 %), antithrombin III (28 %; NR > 70 %) and protein C (14 %; NR > 30 %).

In this clinical context of acute hepatopathy, hypotonia, digestive disease and abnormal hemostasis, a screening for congenital disorder of glycosylation (CDG) was performed using capillary electrophoresis (CE) and western-blot of serum transferrin (Trf). While CE separates Trf glycoforms based on terminal sialic acid content, western-blot allows their detection following molecular weight-based separation [1,2]. Compared to a control (Fig. 1**A**), the CE Trf profile of the patient (Fig. 1**B**) exhibited an increase in both the 2-sialo (3.8 %; N < 1.6 %) and the 0-sialo Trf fractions (2.6 %; N = 0 %). Using western-blot (Fig. 1**C**), Trf migrates into a single protein band in healthy controls (lanes C). In a CDG-I patient (last lane), the partial deficiency of entire *N*-glycans chains results in the emergence of two additional lower bands, alongside the persisting normal one. For the patient (second lane), the profile mirrored that of the CDG patient. Altogether, these results strongly suggested the diagnosis of CDG, and possibly a mannose-phosphate isomerase deficiency (MPI-CDG or CDG-Ib) that commonly presents early in life with severe liver and digestive symptoms [3].

Concurrently, considering the very severe initial clinical phenotype and diagnostic errancy, a whole exome sequencing (WES) was prescribed. DNA was extracted from blood samples from the proband and unaffected parents using the Promega blood DNA extraction kit. The exome was sequenced in the genetics laboratory of the university hospital in Tours using capture (Twist comprehensive Exome, Twist Bioscience) and Illumina sequencing (Nextseq 550, High output kit v2.5 150 cycles). After demultiplexing, alignment on the hg19/GRCh37 reference genome and annotation of variants were carried out on the Seqone bioinformatics platform (SeqOne Genomics). Trio exome analysis found a missense variant (NM_000035.4:c.1013C > T; NP_000026.2:p.(Ala338Val)) at the homozygous state in the *ALDOB* gene in the proband, inherited from both parents. *ALDOB* (chromosome 9) codes for aldolase B, a glycolytic enzyme, expressed in the liver, kidneys, and intestines, which catalyzes the reversible conversion of fructose 1-phosphate (F1-P) into glyceraldehyde and dihydroxyacetone phosphate. The *ALDOB* gene is an OMIM morbid gene associated with hereditary fructose intolerance (HFI) with autosomal recessive inheritance. According to the GnomAD database, allele frequency was 0.0001423 (gnomad.broadinstitute.org, v2.1.1, accessed March 2021). The pathogenicity prediction scores from SIFT (sorting intolerant from tolerant), Polyphen 2 and REVEL (rare exome variant ensemble learner) are all in favor of the pathogenicity of the variant and indeed, it was also

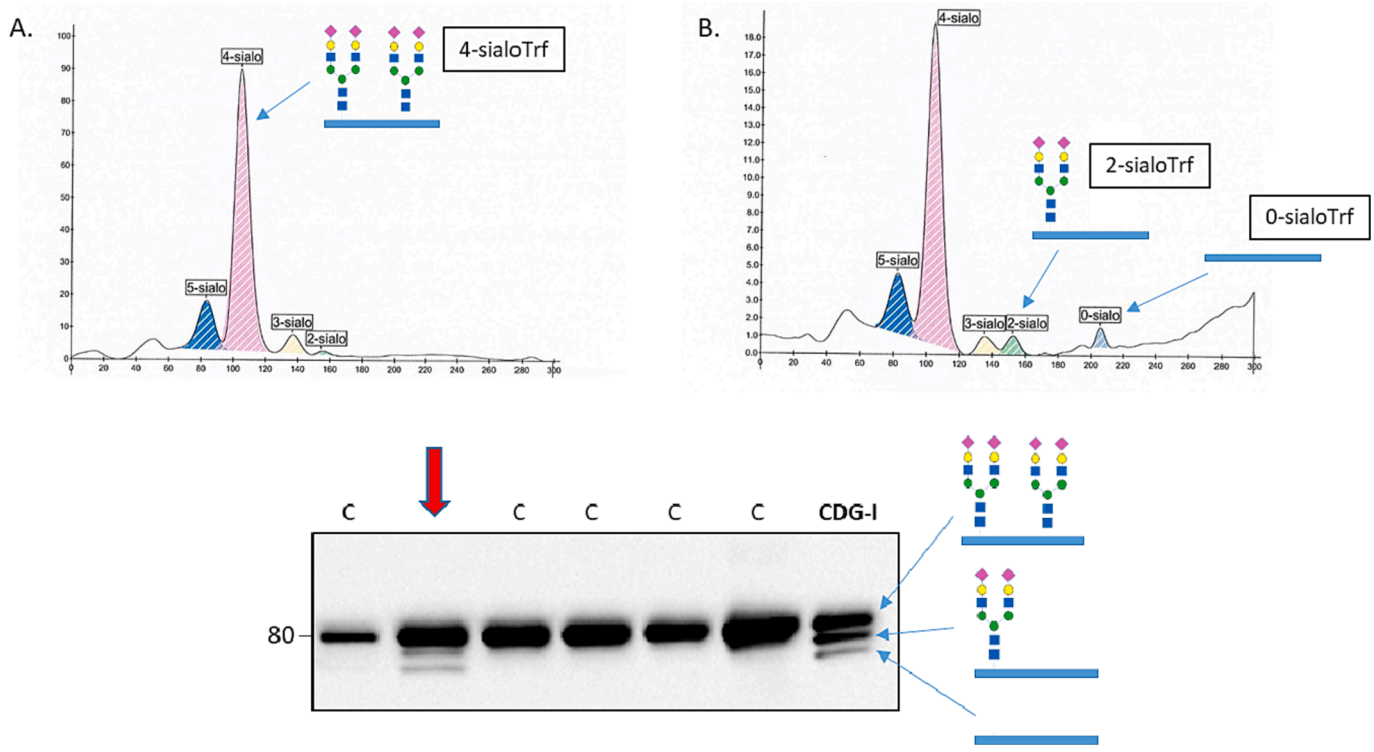

Fig. 1. Capillary electrophoresis (CE) and western-blot of transferrin (Trf) glycoforms, (A) CE Trf profile of a healthy control. (B) Compared to the control, the patient's profile demonstrated an elevated level of both the 2-sialo and the 0–sialo Trf fractions, suggestive for a type 1 CDG (CDG–I). (C) Western-blot of Trf from control samples (C), patient (bold arrow) and CDG-I (far right). Both the patient's profile and that of the CDG-I exhibit two additional bands indicative of the presence of Trf glycoforms lacking complete *N*-glycan chains. Purple diamond: sialic acid; yellow circle: galactose; green circles: mannose; blue square: *N*-Acetyl glucosamine.

reported as pathogenic in the ClinVar database (variation ID: 188739) and in the literature [4–7].

Even if, retrospectively, the symptomatology was compatible with a severe decompensation of HFI, this child was only fed with human milk and formula, both fructose-poor diet not being able to trigger HFI decompensation. After a meticulous medical inquiry of parents, we finally discovered that, having tasted the artificial milk, and finding it "disgusting", they systematically added white sugar (sucrose i.e., fructose-glucose disaccharide) to the baby's milk powder at home. Hence, they unintentionally triggered severe HFI decompensation in their child. In contrast, within the "fructose-free" hospital setting, clinical and laboratory disturbances quickly normalized.

2. Case discussion

Thus, by allowing diagnosis of an atypical case of a treatable inherited disease, WES avoided a potentially fatal erroneous CDG diagnosis. The affected child had HFI that acutely decompensated following iterative parental addition of sucrose into his milk. Owing to his very young age (11-day, and 1-month old) at the times of decompensations, and a supposedly fructose-poor infant diet, the diagnosis of HFI was discarded since we were not aware of the unfortunate parental diet initiative.

HFI or fructosemia (OMIM#229600) is a rare autosomic recessive inherited disease (incidence: 1/30.000 to 1/20.000) linked to aldolase B deficiency resulting in the toxic accumulation of F and deleterious phosphate deprivation in hepatocytes. The first symptoms generally occur at the age of food diversification, classically around 4–5 months of life. Affected individuals may experience severe metabolic crisis usually in the form of abdominal pain, uncoercive vomiting, hypoglycemia or potentially lethal acute hepatitis [8]. Long-term developments mostly consist in severe chronic liver disease with jaundice and hepatomegaly [9]. When correctly diagnosed and treated by dietary fructose, sucrose and sorbitol removal, the prognosis of HFI is excellent. In addition, the cellular accumulation of F1-P has been showed to inhibit the phosphomannomutase 2 (PMM2) enzyme, leading to major impairments in *N*-linked glycosylation mimicking those retrieved in PMM2-CDG. Indeed, together with classical galactosemia (where galactose 1-phosphate accumulates), HFI is a well-known secondary cause of abnormal Trf glycosylation. Therefore routine clinical practice recommende to systematically confirmed CE by genetic analysis [10,11]. Here, this abnormal Trf glycosylation led us to assume a diagnosis of CDG, and in particular of MPI-CDG in the context of early and severe liver disease [3]. This motivated us to consider oral mannose therapy which, besides being ineffective in HFI, would not have triggered the discovery of sucrose addition in the baby's milk by his parents, with the risk of additional and potentially lethal crises.

Major remaining questions concern the cerebral hemorrhage at first admission, as well as the development of a large catheter-located thrombosis at 1 month-old. Can these events be secondary to HFI and ensuing alteration of coagulation? No such early and severe presentation of HFI has already been described considering that patients are generally not exposed to fructose so early in life. At the time of the cerebral hemorrhage, hemostasis parameters were not considered sufficiently disturbed to explain a spontaneous hemorrhage, but their measurement occured after more than 24 h of fructose removal and they thus possibly had already improved. It cannot be excluded that observed bleeding and/or thrombotic manifestations could have resulted from impaired glycosylation of coagulation factors, as it has been extensively described in PMM2-CDG and MPI-CDG [3,12,13].

3. Conclusion

This case highlights the importance of a fluid collaboration between clinicians, biochemists and geneticists when confronted to a severe and atypical presentation of an inherited metabolic disease. Here, both clinical and biological phenotypes could have been explained by a CDG, notably MPI-CDG, and without early WES, we would have erroneously favored this diagnosis. Then, we would not have explored the origin of fructose exposition, with the major risk that parents have continued to give potentially lethal sucrose to their child. Here, genetic analyses have then totally transformed the prognosis of this infant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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