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Case study

Clinical and biochemical features in a Congolese infant with congenital disorder of glycosylation (CDG)-IIx

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ABSTRACT

We describe an infant girl with psychomotor retardation, growth retardation, mild facial dysmorphy, evidence of liver involvement and a type 2 pattern of serum sialotransferrins. Serum transferrin glycan analysis with MALDI-TOF showed an extremely altered *N*-glycan pattern with a large number of truncated asialoglycans pointing to a severely defective *N*-glycan processing. The basic defect in this patient with CDG-IIx has not yet been identified.

1. Introduction

Congenital disorders of glycosylation (CDG) are a large family of genetic diseases due to decreased or increased glycosylation of glycoconjugates.[1–3] Some 29 defects have been identified since Jaeken et al. reported the first patients in 1980.[4] There are four main groups of CDG: diseases of protein *N*-glycosylation, of protein *O*-glycosylation, of combined *N*- and *O*-glycosylation, and of lipid glycosylation. *N*-glycosylation defects can be due to defective assembly (cytosolic and endoplasmic reticulum (ER) defects) or to defective processing (ER and Golgi defects). Clinical features of the different CDG types are heterogeneous and range from severe to mild, and from multisystem to mono-organ involvement, but neurological manifestations are mostly predominant.

Isoelectrofocusing (IEF) of serum transferrin (Tf) is the method of choice to screen for *N*-glycosylation defects accompanied by deficiency of sialic acid, a negatively charged sugar. The abnormal IEF patterns show a cathodal shift and can be grouped into two types: type 1 with a decrease of tetrasialoTf and an increase of disialo- and asialoTf pointing to an assembly defect, and type 2 with an increase also of the

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Abbreviations: CDG, congenital disorder(s) of glycosylation; IEF, isoelectrofocusing; Tf, transferrin
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uneven bands trisialo- and/or monosialoTf, pointing to a processing defect. In the latter case, intermediate and/or abnormal glycans are accumulating, and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) is an important tool in the elucidation of these glycan structures and in the elaboration of a hypothesis about the basic defect.[5,6]

Although most reported patients are Caucasians, patients with very diverse ethnic origins have been described.[7–19] We believe that CDG are underdiagnosed by African clinicians, as their clinical phenotypes can bare similarity to diseases more common in tropical regions.

We report the first black African child with a CDG. Clinical and biochemical data point to CDG-II but the precise defect has not been elucidated.

2. Patient report

This Congolese girl was evaluated at 10 months of age for hypotonia and failure to thrive. She was born to a 37-year-old mother and a 46-year-old father after a 39 weeks uneventful gestation with normal Apgar score. Her birth weight was 3.900 kg (95th percentile), birth length was 50 cm (50th percentile) and head circumference was 35 cm (50th percentile). She was discharged at the age of 10 days after neonatal hyperbilirubinemia and readmitted at the age of 6 months for severe pneumonia treated with Erythromycin.

Fig. 1 – Patient at the age of 16 months. Note hypertelorism and open mouth.

Physical examination at 10 months revealed a weight of 7 kg (3rd percentile), length of 68 cm (3rd percentile) and head circumference of 42.5 cm (3rd percentile). She had axial hypotonia, internal strabismus, hypertelorism, persistently open mouth with tongue protusion, and low-set ears. Her abdomen was soft with firm hepatomegaly (3 cm below the costal margin). She had a developmental delay (psychomotor level of about 2.5 months) and had tic-like head movements. Cardiovascular examination was normal. Skeletal radiography and echocardiography were normal. Electroencephalography showed bilateral temporal waves consistent with epileptiform activity.

The patient continued to show a growth retardation after the first year (at 15 months of age: weight 8 kg (<3rd percentile = 8.9); length 70 cm (<3rd percentile = 76); head circumference 43.5 cm (<3rd percentile = 45) (Fig. 1).

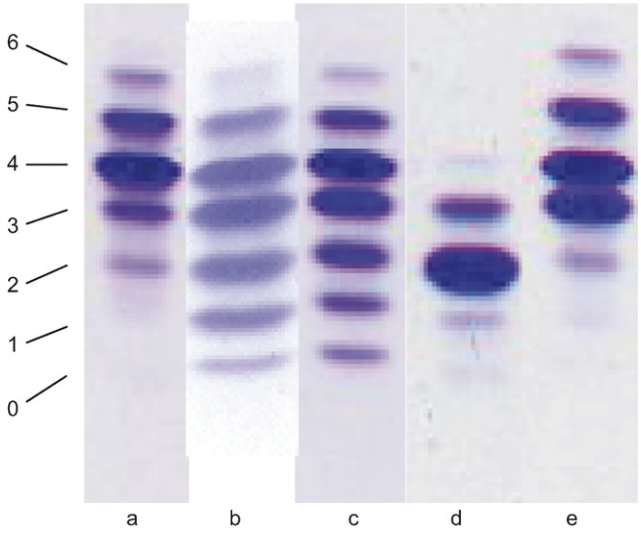

Fig. 2 – Serum Tf IEF pattern in a control (a), in the patient (b) and in three other patients with different type 2 IEF patterns ((c, e): CDG-IIx, (d): CDG-IIa). Numbers on the left indicate sialoTf isoforms.

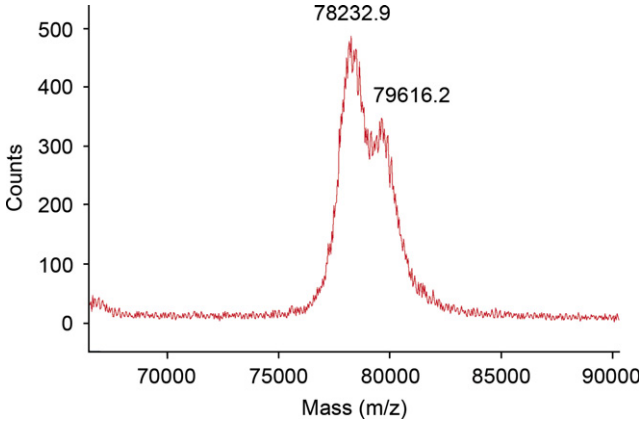

Fig. 3 – MALDI-TOF analysis of intact serum Tf of the patient. The 79616.2 peak represents normal Tf, while the 78232.9 peak is abnormal Tf representing probably truncated asialoTf.

Blood cell count including platelets, as well as glycemia, ammonia levels and serum creatine kinase (CK) were normal. Serum bilirubin (7 mg/dl), glutamate-oxaloacetate transaminase (92 U/l) and glutamate-pyruvate transaminase (80 U/l) were increased while lactate dehydrogenase and albumin were decreased (76.9 U/l; reference range: 228–456 and 2.81 g/dl; Nl: 3.1–3.5, respectively). Thyroid function (T3, T4, TSH) and routine CSF analysis were normal. Coagulation studies, hepatic ultrasound and cerebral computed tomography were not performed.

A fibroblast culture from a skin biopsy was unsuccessful.

She was readmitted 5 months later with severe respiratory distress, seizures, pallor and jaundice resulting from a Klebsiellae pneumoniae infection. She died with a gastric bleeding after a week of intensive care at the age of 19 months. The parents refused a new skin biopsy.

3. Methods

IEF of serum Tf[20] and of apo C-III[21] were performed as described.

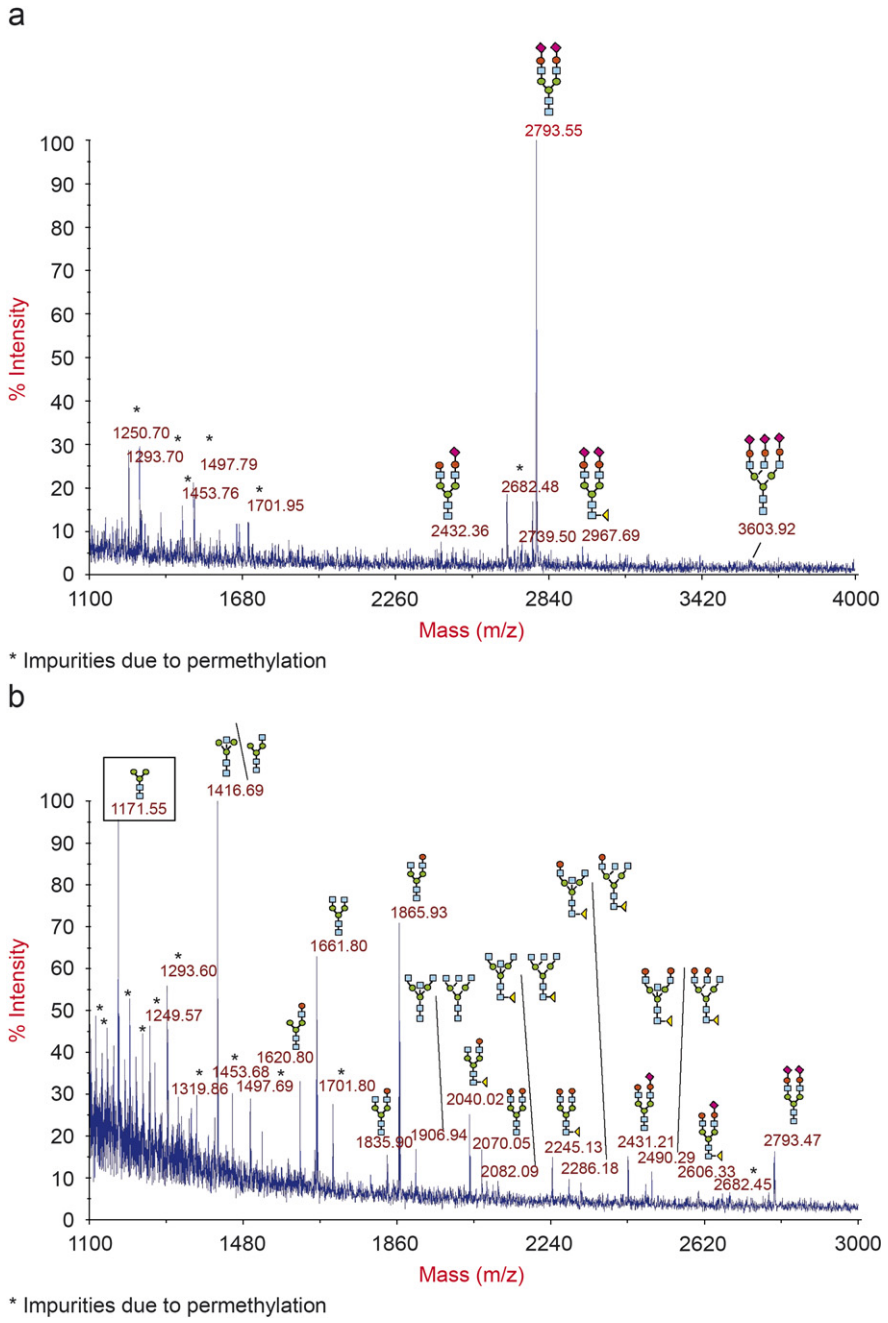

Fig. 4 – (a) MALDI-TOF analysis of permethylated Tf glycans in positive reflector mode in patient: ■: N-acetylglucosamine; ●: mannose; ●: galactose; ◆: sialic acid; ◀: fucose. Note the large number of truncated asialoglycans (including a $GlcNAc_2Man_3$ glycan (boxed)) compared to control (b).

The N-glycans of Tf were released by PNGase F treatment. These N-linked glycans were purified by solid-phase extraction and subjected to permethylation in the presence of sodium hydroxide. The permethylated glycans were then analyzed by MALDI-TOF mass spectrometry (MS) in negative and positive ion mode.[22,23]

The MGAT1 gene was analyzed by direct sequencing.

4. Results

Capillary zone electrophoresis and IEF of serum Tf showed a type 2 pattern with a decrease of tetrasialoTf and increased disialo-, trisialo-, mono- and asialoTf (Fig. 2). IEF of serum Tf after neuraminidase treatment showed only one Tf band excluding a Tf protein variant. IEF of serum apo C-III was normal.

MALDI-TOF analysis of intact serum Tf of the patient showed a prominent abnormal peak suggesting a large amount of truncated asialoglycans (Fig. 3). MALDI-TOF analysis of serum Tf in negative linear mode (not shown) and in positive reflector mode (Fig. 4a and b) confirmed a severely reduced amount of tetrasialoTf and an extremely altered N-glycan pattern with a large number of truncated asialo glycans (including a $GlcNAc_2Man_3$ glycan), pointing to severely defective glycan processing (Sturiale et al., in preparation).

Mutation analysis of MGAT1 did not show any pathogenic mutation at the level of genomic DNA.

5. Discussion

The clinical syndrome of this patient comprised psychomotor retardation, growth retardation, mild facial dysmorphy and evidence for liver involvement. She showed in addition a type 2 serum sialoTf pattern not due to a Tf protein variant. Since IEF of serum apo C-III was normal these data pointed to an N-glycosylation disorder without evidence for impairment of mucin type O-glycosylation. Serum Tf glycan analysis with MALDI-TOF showed a unique pattern not reported before.

CDG with a type 2 serum sialoTf pattern can be divided in CDG with an identified basic defect and CDG with a not yet identified basic defect. There are only few CDG with a type 2 pattern in which the biochemical defect has been identified (Table 1). Among these, CDG-IIa and CDG-IId are N-glycosylation defects[24,25] while the COG7, the COG1 and the COG8 defects are combined N-and O-glycosylation defects.[13,19,33]

On the other hand, there is a rapidly growing group of reported and unreported CDG patients with a type 2 pattern and a still unclear basic defect (CDG-IIx). Among these are the patients with various combinations of rather aspecific symptoms (aspecific dysmorphism, psychomotor retardation, epilepsy, etc.),[26–28] and on the other hand, several clinically recognizable groups such as isolated liver disease,[29,30] cutis laxa syndromes in patients in whom the known cutis laxa defects have been excluded,[31] and a syndrome with glomerulopathy and buphtalmia.[32] The latter syndromes could be excluded in the present patients, and the combined clinical and biochemical data permit us to exclude the elucidated CDG with a type 2 serum sialoTf pattern.

Table 1 – CDG with a type 2 serum Tf IEF and identified basic defect

Disorder	Clinical features	First reported in (Ref.)
CDG-IIa	Psychomotor retardation, epilepsy, stereotype behaviour, dysmorphism	1994[24]
CDG-IId	Psychomotor retardation, Dandy–Walker malformation, myopathy, bleedings	2002[25]
COG7 defect	Encephalopathy, growth retardation, liver disease, hyperthermia	2004[19]
COG1 defect	Mild psychomotor retardation, progressive microcephaly, growth retardation of the rhizomelic type	2006[13]
COG8 defect	Psychomotor retardation, neurological abnormalities (epilepsy, ataxia, etc.), mild dysmorphism	2007[33]

On the basis of the glycan structures, particularly the $GlcNAc_2Man_3$ glycan, we hypothesized that this patient might have suffered from a deficiency of N-acetylglucosaminyltransferase 1 coded by MGAT1. However, MGAT1 analysis failed to show pathogenic mutations.

In conclusion, the present patient is the first reported black African with a CDG. The combined clinical and biochemical data point to a novel (primary or secondary?) CDG, belonging to the CDG-II group (processing defect).

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