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Should *PMM2*-deficiency (CDG Ia) be searched in every case of unexplained hydrops fetalis?

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ABSTRACT

Hydrops fetalis (HF) is characterized by an accumulation of fluid in the extracellular compartments and in body cavities. Non-immune HF (NIHF) is caused by a wide variety of disorders and overall, 20–25% of NIHF remain unexplained. Inborn errors of metabolism, mostly lysosomal storage diseases have been estimated to account for 1–2% of cases, leading to HF by anemia or liver failure. Very few cases of NIHF and Congenital Disorder of Glycosylation (CDG) have been reported. We present here a case of recurrence of HF in a non-related couple in which the diagnosis of CDG type I was suspected at fetal pathological examination then confirmed at the enzymatic and molecular levels, as well as on a characteristic CDG I serum transferrin profile at 30 weeks of gestation. We also provide a systematic review of reported cases with CDG type I and NIHF reported thus far. When NIHF remains unexplained despite exhaustive obstetrical screening, analysis of PMM activity in the parents' leucocytes is possible and might be performed easily during pregnancy. The accurate diagnosis is important in terms of counseling during pregnancy or later, in order to allow an early molecular prenatal diagnosis for the following pregnancies.

1. Introduction

Hydrops fetalis (HF) is defined by the presence of fluid in at least two fetal compartments, including skin thickness>5 mm, pleura, pericardium, or peritoneal cavity. It can be associated with polyhydramnios or hydropic placenta. HF causes are usually slept in immune and non-immune ones. Immune HF results from hemolytic disease mostly due to Rh alloimmunization. Nowadays, thanks to routine immunization of Rh negative mothers, immune HF has become less frequent, accounting for around 20% of HF cases.

Non-immune HF (NIHF) is caused by a wide variety of disorders recently reviewed and classified in over 6000 individuals [1]. Main causes are cardiovascular disorders (21%), anemia (10%), chromosomal disorders (13%), infections (7%), lymphatic dysplasia (6%), thoracic abnormalities (6%), twin-to-twin transfusion (6%) and other more rare etiologies [1]. Overall, 20–25% of NIHF remain unexplained and a significant number may result from an undiagnosed metabolic disease. Among the minor causes, 13 different lysosomal storage diseases can lead to NIHF (review in [2]), and have been estimated to account for 1–2% of all NIHF. The incidence of lysosomal storage diseases in NIHF is as high as 15% in a series where other etiologies are excluded [3]. Among other inborn errors of metabolism, fewer cases of congenital disorder of glycosylation (CDG) with NIHF/ascites have been reported [4–13]. We present here a case of recurrence of HF in a non-related couple in which the diagnosis of CDG Ia was retrospectively suspected at fetal pathological examination and confirmed at the enzymatic and molecular levels, as well as a serum CDG I transferrin profile at 30 weeks of gestation.

2. Material and methods

2.1. Family report

The first pregnancy of a non-related couple was unremarkable with the delivery of a healthy girl.

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Four years later, the second pregnancy was terminated at the 3rd trimester because of severe HF, with severe skin edema, pericardial effusion and ascites. The placenta was hydropic and there was a severe polyhydramnios. Fetal umbilical blood sampling showed thrombocytopenia (platelet count 15×10^9/l), leucopenia (leukocytes: 2.7×10^9/l; 80% lymphocytes; 14% neutrophils) without anemia (hemoglobin concentration 122 g/l, erythrocytes 2.80×10^9/l) and elevated total bilirubin (45 μmol/l), γGT (83 UI/l). Fetal infection, chromosomal anomalies, heart and thoracic malformations, hemochromatosis, and lysosomal storage diseases were excluded. A medical termination of pregnancy was performed at 33.5 weeks and fetal autopsy was performed (Figs. 1a–f). There was dysmorphy including middle face hypoplasia, little nose, frontal bossing (Figs. 1a–b), preauricular tag (Fig.1 c), and inverted nipples (Fig.1 d). Internal findings were hard pancreas and hepatosplenomegaly. Kidneys were macroscopally unremarkable, but histology showed slight renal tubulocystic changes in the cortex and in the medulla without fibrosis or dysplasia (Fig. 1e), and numerous Perls positive iron deposits, often calcified in renal tubules. The liver showed an expanded portal tract with proliferation of irregular ducts, few and non-dissecting fibrosis, and no steatosis nor cholestasis, intense haematopoiesis and intracellular hemosiderin deposition mainly in periportal areas (Fig. 1f–g). The pancreas showed sparse acinar dilatation, endocrine islet hyperplasia, and karyomegaly. Bone marrow was rich but without megakaryocytes. Brain examination showed only edema.

During the 3rd pregnancy, a large nuchal translucency led to a chorionic villi sampling; karyotype was normal. At 22 weeks, a thin pericardial effusion in the 2nd systematic ultrasound scans led to a closer follow-up. In the 25th week, a mild ascites, skin edema and an excess of amniotic fluid were observed in addition. At 29 weeks, a frontal bossing was observed. The placenta thickness was normal. Blood cells count showed thrombocytopenia (platelet count 19×10^9/l) without anemia (Hb: 143 g/l). Unlike the previous sibling, fetal examination showed only one inverted nipple, kidney and liver histology were normal (Figs. 1k–l). CDG was particularly suspected because of the inverted nipples and the kidney and liver histology in the first sibling.

3. Methods

Phosphomannomutase (PMM) activity was measured in leukocyte of both parents [14]. Mutational screening of the *PMM2* gene was performed by direct sequencing of all eight exons and flanking introns from genomic DNA using the primers described previously [15]. Sequences were determined on an ABI PRISM 3100 (Applera). Mutation nomenclature was based on the Human Genome Variation Society (HGSV) web site recommendations. Western blot-based method was used to detect serum transferrin species with reduced molecular masses due to hypoglycosylation [16].

4. Results

Phosphomannomutase (PMM) measurement in the leukocytes of both parents showed a decreased activity. Subsequently, mutational screening of the PMM2 gene identified heterozygous compound pathogenic mutations in the first affected sibling and ongoing pregnancy: a [c.58C>T; c.66+1G>T], [p.Pro20Ser; splice mutation] allele was inherited from the mother and a c.357C>A, (p.Phe119Leu) mutation was inherited from the father. Western blot analysis of serum transferrin in the second sibling showed glycosylation abnormalities characteristic of CDG I [16].

5. Discussion

NIFH is caused by a wide variety of disorders and overall, 20–25% of NIHF remain unexplained. Inborn errors of metabolism are suspected when recurrence occurs or in case of consanguinity suggesting an autosomal recessive disorder. During the second pregnancy, samples from the post mortem examination of the previous sibling were reviewed and several metabolic diseases were discussed. In Pearson syndrome hydrops, hepatic fibrosis, duct proliferation, renal anomalies and thrombocytopenia with or without anemia can be observed; however, characteristic cytoplasmic

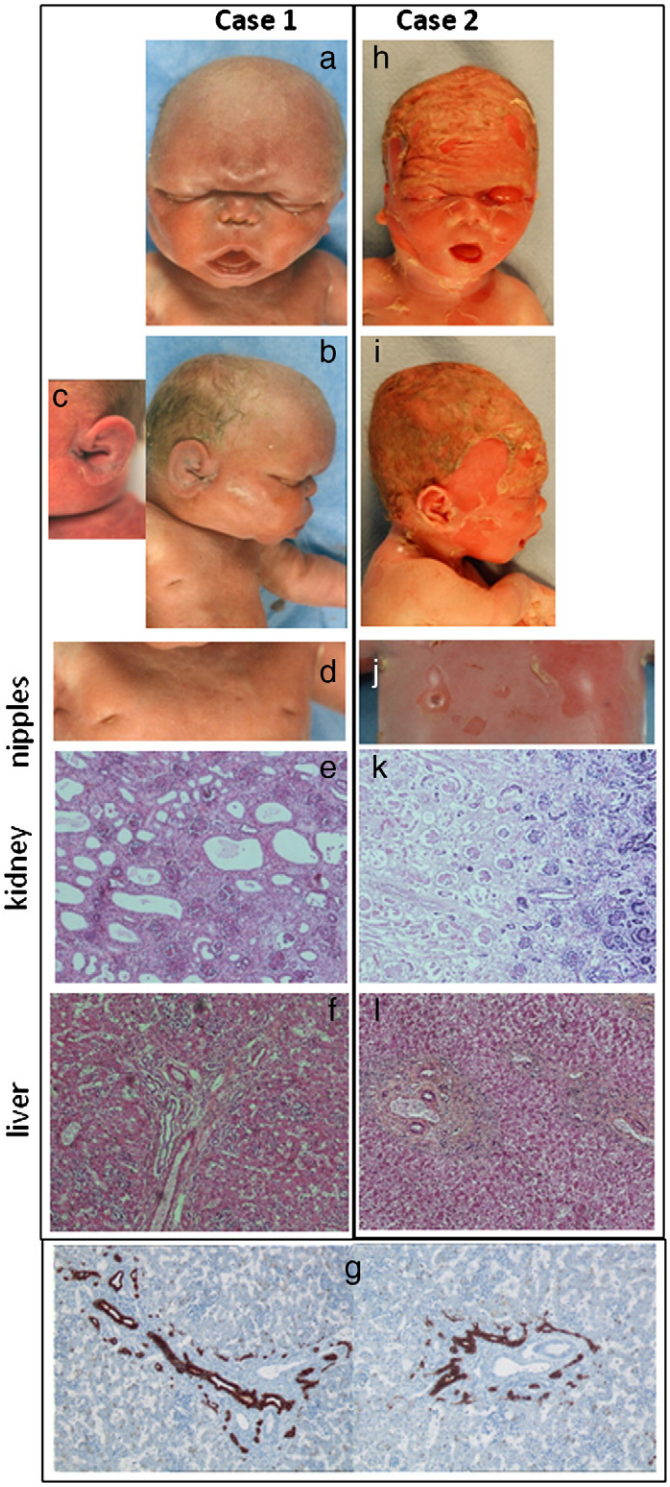

Fig. 1. Fetal pathology findings of first (a–g) and second (h–l) affected siblings. a,b,h,i, dysmorphic features of both cases note hydrops, frontal bossing, midface hypoplasia; little nose. c, unilateral preauricular tag. d,j, inverted nipples. e, k, histology: renal tubulocystic changes. f, l, histology: expanded portal tract with proliferation of irregular ducts in liver, better shown by cytokeratine 7 immunostaining (g).