Virchows Arch (2000) 437:101–105

CASE REPORT

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Congenital hepatic fibrosis in 3 siblings with phosphomannose isomerase deficiency

Received: 19 July 1999 / Accepted: 12 January 2000

Abstract Congenital hepatic fibrosis is a rare disorder of intrahepatic bile ducts with the persistence of embryological bile duct structures in ductal plate configuration. Three siblings aged 18, 17 and 14 years old were found to have congenital hepatic fibrosis associated with a deficiency of the enzyme phosphomannose isomerase. The clinical symptoms were recurrent attacks of persistent vomiting with diarrhea and mild hepatomegaly. The biochemical abnormalities included elevated serum transferases during attacks, clotting factor deficiencies and persistent hypoalbuminemia. In the youngest patient protein-losing enteropathy was present. Liver biopsies of the three patients taken when they were 1, 3 and 14 years old showed an excess of bile duct structures in ductal plate configuration with mild fibrosis in the portal triads. In one patient the liver biopsy was repeated after 18 years and showed only a mild progression of fibrosis in the portal triads. Duodenal biopsies taken in infancy in two of the three patients did not show any abnormalities. Recognition of phosphomannose isomerase deficiency in association with congenital hepatic fibrosis and protein-losing enteropathy is important, because some of the clinical symptoms are potentially treatable by oral mannose therapy.

Key words Congenital hepatic fibrosis · CDG syndrome · Phosphomannose isomerase deficiency

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Introduction

Congenital hepatic fibrosis (CHF) is a rare disorder of intrahepatic bile duct development associated with ductal plate malformation. Ductal plate malformation is the morphological description of the persistence of an excess of embryological bile duct structures in ductal plate configuration [7]. CHF has been reported as an isolated defect, but is frequently associated with autosomal recessive polycystic kidney disease [1]. CHF has also been reported in association with many malformation syndromes and liver disorders, such as Caroli's disease and choledochus cysts [8]. The clinical manifestations of congenital hepatic fibrosis may vary from relatively few clinical symptoms to severe portal hypertension and/or recurrent episodes of cholangitis [2, 8, 22]. The histological findings in CHF can also vary, and include enlarged portal tracts with bile ducts in ductal plate configuration and bands of connective tissue containing ductal plate remnants. The abnormalities may be aggravated with increasing fibrosis or remain relatively unchanged for a long period [9]. We report a familial form of CHF in which the leading symptoms included cyclic vomiting with diarrhea and mild hepatomegaly associated with clotting factor deficiencies and protein loss in the gut. The affected family members were found to have a deficiency of the enzyme phosphomannose isomerase (PMI). This disorder was recently diagnosed as a new inborn error of protein glycosylation [16, 19]. The defect belongs to the relatively new and rapidly expanding group of the carbohydrate-deficient glycoprotein (CDG) syndromes. These diseases are severe multisystem disorders, first reported by Jaeken et al. [12]. Except for the here described PMI deficiency, the CDG syndromes are charac-

terized by dysmorphism, extensive neurological dysfunction, multiorgan involvement and abnormalities of circulating proteins. At least six subtypes have been recognized, the most frequent of which is CDG syndrome type IA, or phosphomannomutase deficiency [13, 14, 16, 17, 23, 24]. Recognition of congenital hepatic fibrosis and protein-losing enteropathy associated with PMI deficiency is important, as some of the clinical symptoms are potentially treatable with mannose therapy [18, 19].

Clinical history

Some of the biochemical and the clinical findings in this family have been reported previously [16]. The three patients, two females and one male, were born to healthy consanguineous Turkish parents. A baby girl had died of unknown cause at the age of 3 months and a younger girl was unaffected. At the time of diagnosis the patients were 18, 17 and 14 years old. They all presented identical clinical symptoms starting in the 1st year of life, with recurrent attacks of vomiting accompanied by diarrhea and dehydration. They had mild hepatomegaly, which disappeared in adolescence. On repeated renal ultrasound no abnormalities were found indicating polycystic kidney disease. Mental and motor development were completely normal. Routine laboratory investigations showed iso- or hypotonic dehydration during disease episodes with moderate elevated serum transaminases and persistently mild to moderate hypoalbuminemia (Table 1). A defect in protein glycosylation was suspected from the predominance of asialo- and disialotransferrin isoforms on serum transferrin isoelectric focusing.

This diagnosis was confirmed by the demonstration of a deficiency of the enzyme phosphomannose isomerase in liver tissue, cultured skin fibroblasts and lymphocytes [16]. The three patients were reinvestigated, as other authors reported protein-losing enteropathy and clotting factor deficiencies in patients with the same enzyme defect [15, 19]. Protein loss was only present in the youngest patient and had not been detected before, but clotting factor deficiencies were present in all three patients (Table 1). Patient 2 was found to be suffering from a chronic persistent hepatitis B infection at the age of 19 years. Mannose therapy was initiated in the oldest patient but was discontinued due to poor compliance.

Materials and methods

Liver biopsies were performed in patient 1 at 3 years of age, and in patient 2 at 1 year of age, repeated at 19 years of age. In patient 3 a liver biopsy was performed at 14 years of age. Duodenal biopsies were done in patients 2 and 3 when they were 3 and 4 years of age, respectively. On light microscopy the duodenal biopsies were normal. There was no archival material available for additional electron microscopy. The biopsies for light microscopy were routinely fixed in formalin and embedded in paraffin. Sections 4 µm thick were stained with hematoxylin and eosin, Masson trichrome, PAS after diastase digestion, iron and rhodamine copper stain. For immunohistochemical examination tissue sections were stained with antibodies against hepatitis B core and surface antigen (Dako) using the avidin-biotin-peroxidase method. Small parts of the liver biopsies were fixed in Karnovsky (formalin and 2% glutaraldehyde) and postfixed in 2% osmium tetroxide and embedded in Araldite. Ultrathin sections were stained with lead citrate and uranyl acetate.

Pathological findings

Light microscopy of liver biopsies

All three patients showed ductal plate malformation in their biopsies (Figs. 1–3). The liver architecture with portal triads and alternating central veins remained intact. The portal triads were enlarged, with an increase of large round to oval bile duct structures. Some of the enlarged bile ducts were located in the periportal area, and some bile ducts were situated in the center of the portal triad. A single layer of cuboidal epithelium lined the bile ducts and there was no bile stasis (Figs. 1A, 2, patient 2, 1 year of age). In the biopsies taken early in life (1 and 3 years of age) some porto-portal connections were observed and there was some fibrosis (Fig. 1B, patient 2). It is suggested that later in life the portal triads were larger, with more and dense connective tissue. There was a slight increase in parenchymal fibrosis surrounding individual hepatocytes (Fig. 3A, B, patient 3 at 19 years of age). Bridging fibrosis between portal triads and central veins was not observed. There were no signs of regeneration. The hepatocytes contained no lipid, iron, copper or bile. The wedge biopsy from patient 2 at 1 year of age showed a mild mononuclear infiltrate with an occasional neutrophilic granulocyte. In the biopsy at 19 years of age a slight increase of portal mononuclear infiltrate was seen and some hepatocytes stained positive with monoclonal antibodies against hepatitis B core and surface antigen. The increased amount of bile duct-like structures in this biopsy stained positive with monoclonal antibodies reacting against cytokeratin 7; there was no archival material left from this and the other patients to do additional immunostaining.

Table 1 Abnormal laboratory findings in serum and feces of three patients with phosphomannose isomerase deficiency. Albumin was persistently low, aminotransferases were only elevated during attacks. Clotting factors and α-1 antitrypsin in feces were only investigated in symptom-free periods (*ASAT* aspartate aminotransferase, *ALAT* alanine aminotransferase, *u/l* units per liter)

	Mannose (nmol/l)	Albumin (g/l)	ASAT (u/l)	ALAT (u/l)	Antithrombin III (%)	Protein-C (%)	Protein-S (%)	α-1 Antitrypsin in feces (mg/g)
Patient 1	21–29	19–34	42–493	45–403	44	45	50	<0.8
Patient 2		30–34	46–182	45–191	81	65	77	<0.8
Patient 3		23–32	27–108	16–218	43	51	43	Max. 8
Normal	45–65	35–45	15–35	15–35	90–110	76–159	66–126	0.0–1.7

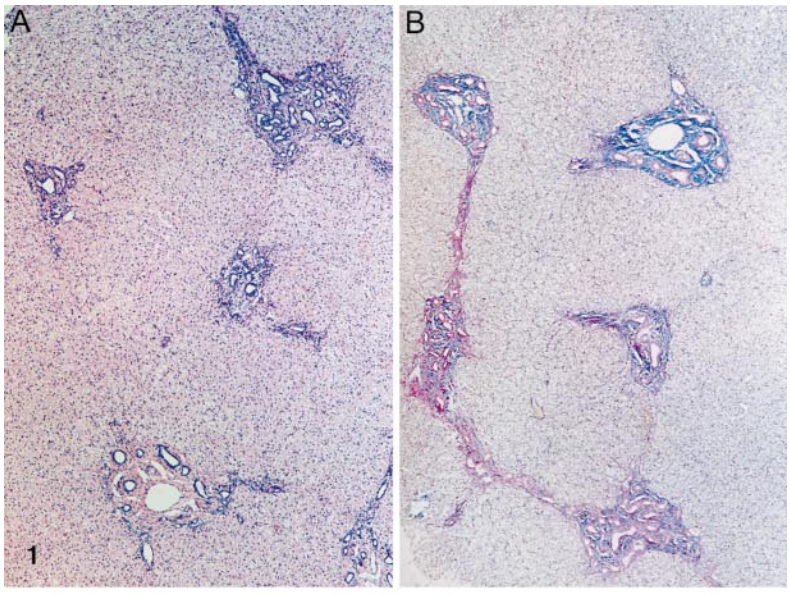

Fig. 1A, B Wedge biopsy from patient 2 at 1 year of age. The ductal plate malformation with fibrosis and porto-portal connections is clearly seen. **A** H and E, ×50; **B** trichrome, ×50

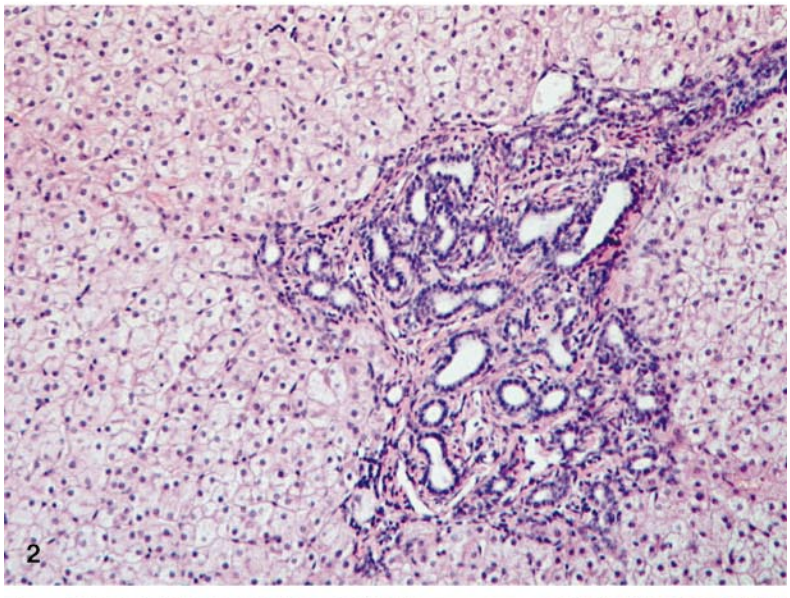

Fig. 2 Magnification of one portal triad from the wedge biopsy from patient 2 at 1 year of age. Note the increased amount of round to oval bile duct-like structures. A single layer of cuboidal epithelium lined the bile ducts, and there was no bile stasis. H and E, ×260

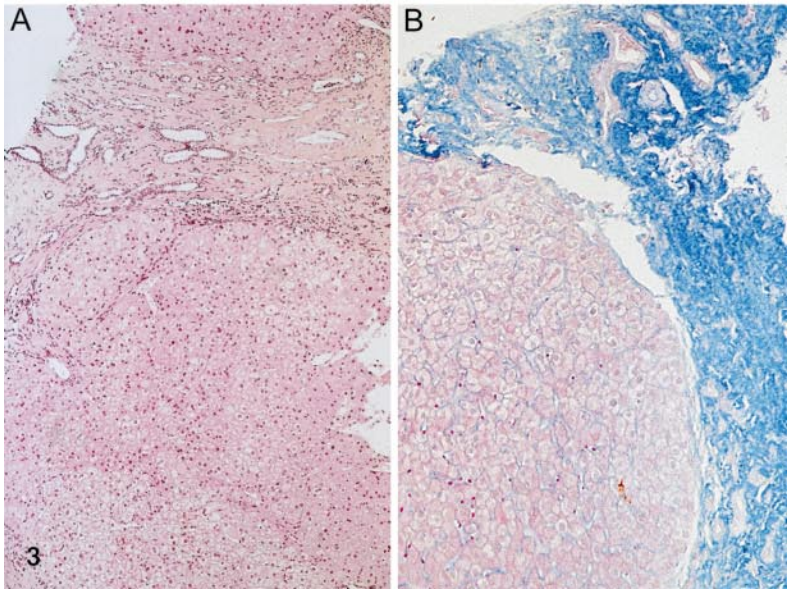

Fig. 3A, B Needle biopsy from patient 2 at 19 years of age. Ductal plate malformation with dense connective tissue in portal triads and slight increase of parenchymal fibrosis is seen. There is some increase of mononuclear infiltrate. **A** H and E, ×90; **B** trichrome, ×150

Electron microscopy.

Ultrastructural analysis of a liver biopsy from patient 3 at 14 years of age showed a slight increase of collagen surrounding individual hepatocytes. The only apparent abnormality in the hepatocytes was a slight increase of the Golgi apparatus.

Discussion

The histological abnormalities present in all three patients were consistent with a diagnosis of congenital hepatic fibrosis. An excess of bile duct structures in ductal plate configuration was found in all biopsies. The histological abnormalities were mild in all three patients, which is consistent with the absence of severe clinical manifestations such as portal hypertension or recurrent episodes of cholangitis. The biopsies taken at young ages in patients 1 and 2 showed little fibrosis and slightly enlarged portal triads. The biopsy in patient 3 taken at 14 years showed more fibrosis in the portal triads compared to the biopsies taken of his sibs in infancy. There was some progression of the histological abnormalities over time, as seen in patient 2 in whom the liver biopsy was repeated after 19 years. However, this patient was also affected by a hepatitis B infection. The histological findings in this family were no different from the hepatic findings reported in other disorders associated with CHF [8, 9], and therefore the diagnosis of PMI deficiency should be suspected on the basis of the specific clinical and biochemical abnormalities.

We are not the first to report a familial occurrence of congenital hepatic fibrosis associated with protein-losing enteropathy and clotting factor abnormalities. This particular combination of symptoms was first recognized by Pedersen and Tygstrup [21]. In their original paper, two siblings were reported with recurrent thrombotic events, hypoalbuminemia due in part to gastrointestinal protein loss and congenital hepatic fibrosis. It is not unlikely that these patients indeed suffered from PMI deficiency as in our patients. More recently congenital hepatic fibrosis has been reported in other patients with PMI deficiency, but no details on the liver histology were presented [11, 15, 18].

PMI deficiency affects the biosynthesis of oligosaccharide chains of proteins. The synthesis of the oligosaccharide chains of glycoproteins is a complex process, starting with the assembly of an oligomannose structure. These mannoses originate from mannose-6-phosphate (Fig. 4). Phosphomannose isomerase catalyzes the conversion of fructose-6-phosphate to mannose-6-phosphate. In PMI deficiency the formation of mannose-6-phosphate is compromised, and this will subsequently lead to reduced *N*-glycosylation of proteins. However, mannose-6-phosphate can still be formed from mannose through hexokinases offering a potential treatment by bypassing the metabolic defect [18, 19]. A reduced *N*-glycosylation of proteins can be demonstrated by an excess of hypoglycosylated isoforms of serum transferrin on isoelectric focusing. Transferrin isoelectric focusing in this family showed a predominance of asialo and disialo isoforms, a pattern that is biochemically indistinguishable from the most frequent CDG syndrome type IA. For this reason PMI deficiency has been catagorized as CDG syndrome IB [19]. In the majority of CDG patients with a type I isoelectric focusing pattern a deficiency of the enzyme phosphomannomutase (PMM), or CDG syndrome type IA, is present [14]. This enzyme catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate, the next step after PMI in the early mannose pathway (Fig. 4). Although PMI deficiency is biochemically indistinguishable from PMM deficiency, the clinical manifestations and the histology of associated liver abnormalities are very different. Conradi et al. [6] describe the histological findings in liver biopsies of CDG type IA patients. On light microscopy, fibrosis was present within lobules and as bridges between portal fields. There were no signs of hepatic regeneration. Other findings included a mild monocellular infiltration in portal fields and the hepatocytes contained granular material stained by PAS and lipid vacuoles which appeared empty in paraffin-embedded material. Electron microscopy showed remarkable abnormalities with numerous lysosomal inclusions with concentric electron-dense membranes and variable eletron-lucent and electron-dense material. The ultrastructural abnormalities in these biopsies were similar to those found in Niemann-Pick type C and were also reported to be present in sural nerve biopsies of patients with CDG syndrome type IA [20]. The histological abnormalities were not progressive in repeated liver biopsies. None of these specific histological findings or ultrastructural abnormalities were present in the liver biopsies from our patients with PMI deficiency. Ultrastructural abnormalities of small bowel were reported in PMI deficiency by other authors. Niehues et al. [19] found an enlarged endoplasmatic reticulum with long tubular bundles in small bowel samples of a young PMI deficient patient with severe protein-losing enteropathy.

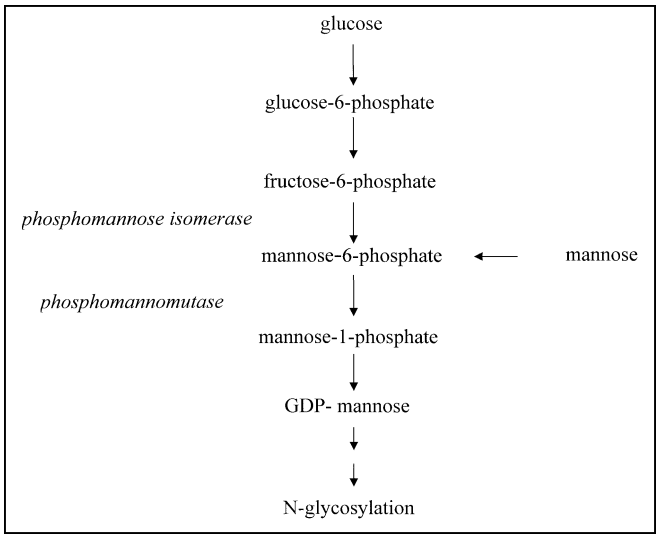

Fig. 4 The early mannose pathway

The pathogenesis of ductal plate malformation was recently discussed in an excellent review [9]. This very complex developmental process of intrahepatic bile duct formation is accompanied by the expression of a number of proteins by hepatoblasts differentiating to biliary structures. The proteins involved include cytokeratins, tissue polypeptide antigen, biliary glycoprotein I, carcinoembryonic antigen, epithelial membrane antigen and carbohydrate antigens [3, 4, 9, 10, 25] Furthermore, epithelial proliferation and apoptosis are involved in remodeling of the ductal plate [9, 26]. Many of the proteins involved are indeed glycoproteins, although not all of them have *N*-linked oligosaccharide chains [5].

It is attractive to speculate that hypoglycosylation of proteins is involved in the altered developmental patterns observed in CHF associated with PMI deficiency. Why, in contrast to the other CDG syndromes, the abnormalities in PMI deficiency are restricted to the liver and gut is not clear at the moment.

In conclusion, we report the histological findings of CHF in 3 siblings with a deficiency of the enzyme PMI. Although congenital disorders of intrahepatic bile ducts are usually not associated with inborn errors of metabolism, we strongly recommend that PMI deficiency is considered in the differential diagnosis of CHF, especially in those patients with clotting factor abnormalities and/or protein loss in the gut.

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