

• CASE REPORT •

# Liver cirrhosis as a consequence of iron overload caused by hereditary nonspherocytic hemolytic anemia

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## Abstract

Nonspherocytic hereditary anemias are occasionally accompanied by significant iron overload but the significance for the development of chronic liver disease is not clear. We described two cases of patients with chronic liver disease and severe iron overload due to chronic hereditary hemolysis. Both patients have had signs of liver cirrhosis and severe hemolysis since childhood. A hereditary pyruvate kinase deficiency (PKD) was discovered as the underlying reason for the hemolysis. Sequencing of the pyruvate kinase gene showed a mutation within exon 11. Liver histology in both patients revealed cirrhosis and a severe iron overload but primary hemochromatosis was excluded by HFE-gene analysis. An iron reduction therapy with desferrioxamine led to significant decrease of serum ferritin and sustained clinical improvement. PKD-induced hemolysis may cause severe iron overload even in the absence of HFE-genotype abnormalities. This secondary iron overload can lead to chronic liver disease and cirrhosis. Therefore, the iron metabolism of PKD patients has to be closely monitored and iron overload should be consequently treated.

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**Key words:** Hemochromatosis; Pyruvate kinase deficiency; Liver cirrhosis; Iron overload; Desferrioxamine

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## INTRODUCTION

Hereditary hemolytic anemia is classified as spherocytic anemia and nonspherocytic anemia. Spherocytic hemolytic anemia is based on an impairment of the erythrocyte membrane or mutations of integral compounds or the hemoglobin molecule. Nonspherocytic hemolytic anemia is caused by a

functional defect of particular enzymes of the erythrocytic glucose metabolism, namely the glycolytic cascade. Since, glycolysis is the only way for mature erythrocytes to generate ATP, inhibition of this pathway results in disturbance of the erythrocytic energy metabolism and, ultimately, hemolysis<sup>[1]</sup>.

Here, we report two cases of patients with severe, nonspherocytic hemolytic anemia caused by hereditary pyruvate kinase deficiency (PKD). The chronic hemolysis led to secondary hepatocellular iron overload and subsequently liver cirrhosis. These results suggest a consequent control of the iron metabolism and the liver function in patients with PK deficiency and, if necessary, an iron reducing therapy.

## CASE REPORT

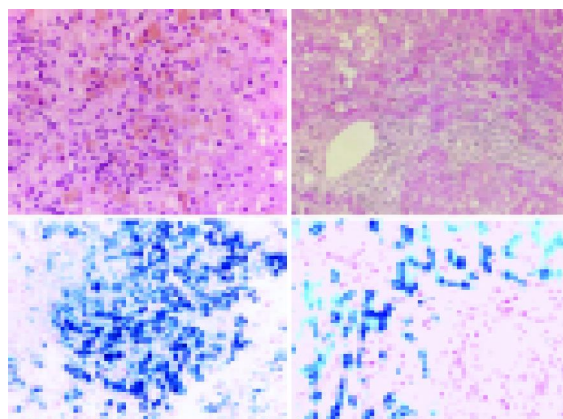
### Case 1

A 56-year-old male patient (patient 1) with severe abdominal pain, fever, nausea and emesis was admitted to the emergency room. In addition, the patient had stigmata of chronic liver disease such as icterus, spider naevi, dilated abdominal subcutaneous veins and ascites. The clinical picture and an abdominal ultrasound examination suggested advanced liver cirrhosis and acute cholecystitis in the presence of gallstones. Laboratory parameters at admission confirmed severe inflammation. A significant iron overload, as indicated by high serum iron and ferritin and siderinuria, was found. Bilirubin was high and the liver function parameters (cholinesterase, prothrombin time and albumin) were significantly altered. However, further differentiation of the elevated bilirubin revealed that the major proportion was indirect bilirubin (>70%). In conjunction with an elevated lactate dehydrogenase (LDH 450 U/L) this was suggestive for hemolysis. Concomitantly, the patient had anemia (hemoglobin 9.4 mg/dL) with significantly stimulated erythropoiesis, as demonstrated by the elevated reticulocyte count of 21% (Table 1).

The patient reported the first onset of icterus at the age of 12. At that time the spleen was removed and he had significant and long-term improvement of the clinical situation. Importantly, the patient revealed a positive family history with a brother (patient 2) who suffered from chronic hemolysis as well. Therefore, further efforts attempting to clarify the etiology of the hemolytic anemia focused on the hereditary hemolytic syndromes. Normal hemoglobin electrophoresis and negative testing for sickle cell disease excluded hemoglobin abnormalities and no spherocytosis or elliptocytosis was found. Osmotic resistance of the red blood cells was normal. However, examination of intracellular erythrocyte enzymes revealed a massively decreased activity of pyruvate kinase

(3.5 U/10<sup>11</sup> erythrocytes; normal: 17.1-26.5), while the activities of glucose-6-phosphate dehydrogenase, hexokinase and glutathione reductase were normal. This finding led to the diagnosis of hereditary PKD.

After the antibiotic pretreatment, open cholecystectomy was performed. The gallbladder was severely inflamed and the intraoperative inspection and palpation of the liver were suggestive for liver cirrhosis. The liver was biopsied and histology confirmed advanced periportal bridging. Furthermore, a severe iron overload of hepatocytes and biliary epithelial cells was present, suggestive for genetic hemochromatosis (Figure 1). However, analysis of the *HFE*-genotype revealed no mutations and other chronic liver diseases, such as alcohol, viral, or autoimmune-related hepatitis and biliary diseases were excluded. Since a causal treatment of PKD is at present not available, an iron-chelating therapy with subcutaneous administration of desferrioxamine twice a day<sup>[2]</sup> was initiated. Despite a significant stabilization of the clinical condition under therapy (no ascites was found in follow-up visits after 3, 6 and 12 mo) and in view of the advanced stage of the liver cirrhosis (Child-Pugh Score 9), the patient was evaluated and recommended for liver transplantation. Unfortunately, he refused transplantation and conservative therapy with desferrioxamine was continued. Unexpectedly, liver function is to date stable for more than 3 years.

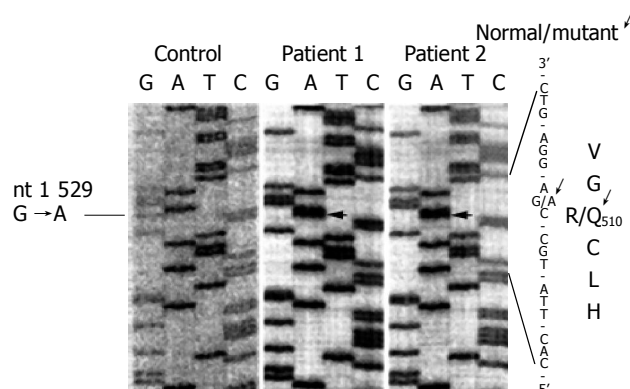


**Figure 1** Liver histology of two patients with hereditary corpuscular hemolysis. Liver biopsies from patient 1 and 2 were fixed in formaldehyde and embedded in paraffin following standard protocols. Sections of 4- $\mu$ m thickness were stained either with hematoxylin-eosin (HE-stain) or Perls Prussian Blue. Hepatic iron deposition was graded according to DiBisceglie.

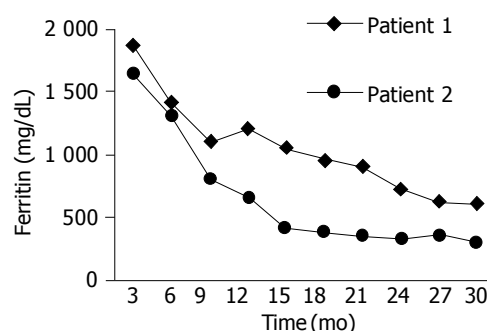
## Case 2

The diagnosis of PKD prompted us to examine the younger brother of the patient, who had a very similar medical history of the development of icterus and concomitant splenectomy during childhood. His actual laboratory parameters are shown in Table 1 (patient 2). The bilirubin elevation in this patient was caused solely by hemolysis, since direct bilirubin proportion was within the normal range (0.7 mg/dL). Correspondingly, all other hemolysis parameters were elevated and erythropoiesis was significantly stimulated

(reticulocyte count of 22%). Pyruvate kinase activity was even lower in this patient than in the older brother. The definitive confirmation of the congenital defect in both patients was now performed by sequence analysis of the pyruvate kinase gene within chromosome 5. A mutation of a single base at position 1 529 within exon 11 of this gene, with substitution of guanine (G) by adenosin (A) was evident (Figure 2). On the amino acid level this mutation could cause an exchange of arginine 510 into glutamate. This patient also had elevated liver enzymes but, in contrast to the older brother, he had no clinical signs of cirrhosis and his liver function was completely normal. There was no evidence for alcoholtoxic, viral or autoimmune hepatitis or an underlying biliary disease. However, iron metabolism parameters with high serum iron and ferritin were in a similar range as in his older brother, indicating massive iron overload. Liver biopsy confirmed the severe hepatocellular iron overload, and revealed furthermore advanced periportal bridging (Figure 1). This patient showed a markedly reduced ejection fraction in echocardiography, indicating iron-induced toxic cardiomyopathy. An iron chelating therapy with desferrioxamine was initiated and complemented by phlebotomy with a bimonthly withdrawal of 500-mL blood. In both patients, the iron reducing therapy led to a significant reduction of iron and ferritin over the next 12 mo (Figure 3).



**Figure 2** Partial sequence analysis of the pyruvate kinase gene (exon 11) in the patients described. Genomic DNA was prepared from peripheral blood mononuclear cells (PBMC) and sequenced according to standard protocols, applying [ $\alpha^{35}$ S] dATP during the termination reaction and subsequent fluorography.



**Figure 3** Decrease of iron overload in the patients under desferrioxamine therapy. Iron metabolism was assessed by serum ferritin concentration over a period of time of 30 mo.

**Table 1** Laboratory parameters of the described patients at admission

Liver inflammation and function	Normal	Patient 1	Patient 2
ALT (GOT),	5–20 U/L	30 U/L	31 U/L
AST (GPT)	5–25 U/L	39 U/L	41 U/L
$\gamma$ -GT	5–30 U/L	41 U/L	61 U/L
Alkaline phosphatase	<180 U/L	224 U/L	219 U/L
Prothrombin time	70–110 %	48 %	102 %
INR		1.43	0.99
Albumin	(3.6–5.0 g/dL)	2.9 mg/dL	4.4 mg/dL
Cholinesterase	(3.5–7.0 kU/L)	2.1 kU/L	4.8 kU/L
C-reactive protein	<0.7 mg/dL	10 mg/dL	0.5 mg/dL
Erythrocyte morphology		anisocytosis, poikilocytosis, acanthocytosis	macrocytosis, anisocytosis, poikilocytosis
Blood count, parameters of hemolysis and iron metabolism			
· WBC	(3–10/nL)	24 /nL	13 /nL
· Hemoglobin	(13.5–16 g/dL)	9.2 g/dL	11.5 g/dL
· Free hemoglobin	(35–65 mg/dL)	150 mg/L	115 mg/L
· Total bilirubin	(0.4–1.0 mg/dL)	8.3 mg/dL	7.6 mg/dL
· Direct bilirubin	(0.2–0.5 mg/dL)	1.7 mg/dL	0.8 mg/dL
· Haptoglobin	(<0.23 g/L)	<0.233 g/L	<0.233 g/L
· Lactat-dehydrogenase	(10–150 U/L)	450 U/L	375 U/L
· Reticulocytes	(<1 %)	21 %	22 %
· Ferritin	(15–630 ng/mL)	1850 ng/mL	1620 ng/mL
· Urine iron excretion		0.5 mg/24 h	0.3 mg/24 h

## DISCUSSION

The diagnosis of PKD in both cases was confirmed by sequence analysis of the PK gene, which revealed a single nucleotide mutation (G to A) in the PK gene within exon 11 at position 1 529. Together with the glucose-6-phosphate-dehydrogenase (G6PD) deficiency, PK deficiency is the most common type of hereditary enzyme defect within the glycolytic pathway causing nonspherocytic anemia<sup>[3,4]</sup>. The point mutation detected in our 2 patients (G1529A) is one of the most frequent mutations within the western European population<sup>[5–7]</sup>. The remaining activity of PK in patients carrying this mutation has been reported to be 10–25% and reticulocytosis varies from 5% to 66%<sup>[7]</sup>. The disease shows a broad range of clinical severity, which is loosely correlated to the impairment of hematological parameters: The lower the residual PK activity and the higher the reticulocyte count, the more the patients are affected<sup>[7,31]</sup>. Since PK catalyzes the final step within the glycolytic pathway (phosphoenolpyruvate→pyruvate), a decreased activity results in alterations of the erythrocellular energy metabolism. Subsequently, the activity of the membrane associated sodium-potassium ATPase is impaired, leading to instability of red blood cells<sup>[8]</sup>.

A main clinical feature in the two PKD patients, examined in this study, is a considerable iron overload. The iron overload is independent of transfusion therapy. In the liver tissue, the iron overload includes hepatocytes and even biliary epithelial cells. Since this histological pattern correlates to genetic hemochromatosis, the HLA phenotype and the commonly known mutations in the hereditary hemochromatosis (*HFE*)-gene (C282Y and H63D) were examined. The possibility of a coincidence of both diseases seems not unlikely, since 2–20% of the European population have mild

alterations of the iron status associated with heterozygosity for the main hemochromatosis mutation (C282Y), while a much higher proportion carry the H63D mutation. A recent study revealed abnormalities of the *HFE*-gene in 35% of nontransfused patients with PKD and these abnormalities coincide with increased serum iron and ferritin<sup>[9]</sup>. However, in our patients heterozygosity for genetic hemochromatosis was ruled out by a normal *HFE*-genotype.

In addition to heterozygosity for hemochromatosis, splenectomy has been reported to be an independent risk factor for iron overload in hemolytic diseases<sup>[10,11]</sup>. While the increased iron turnover is the basis for the iron overload in PKD<sup>[9,12]</sup>, it alone is not sufficient to cause iron accumulation in this disease<sup>[9,12]</sup>. Since both of our patients underwent splenectomy during childhood, this circumstance was considered as a main contribution to the significant iron overload they had. It is well accepted, that primary and secondary iron overloads have prefibrogenic effects within the liver tissue, by sustained induction of oxidative stress<sup>[13,14]</sup>. As in our patients, the level of the liver transaminases may only be marginally elevated, since the iron-induced chronic inflammation is often of very low activity, but nevertheless results in progressive fibrosis<sup>[13]</sup>. Independent of the pathogenesis, liver iron concentration must exceed a critical threshold, which has been suggested to be around 350–400  $\mu\text{mol/L/g}$ , in order to cause hepatocellular damage<sup>[15,16]</sup>. Above this threshold, the secondary iron overload (e.g., due to hemolytic disease) may have the same deleterious effects as the primary iron overload in genetic hemochromatosis.

Taken together, it becomes obvious that the clinical symptoms of the patients described in this study, are all based on the PKD in association with the splenectomy. Under this circumstance, chronic hemolysis is capable of causing significant iron overload, which in turn is responsible for the liver and cardiac disease of both patients. The pathophysiological importance of iron overload in these patients was also demonstrated by the effectiveness of the iron chelating therapy, which led to a significant decrease of serum ferritin and a stable liver function for more than 3 years. In addition to the iron overload, PKD-induced hemolysis is responsible for the abdominal symptoms of the elder patient (case 1). The chronic excess of bilirubin obviously altered the solvent properties of the bile, resulting in cholelithiasis, presumably consisting of bilirubin stones. The stones caused cholecystitis, perhaps combined with acute or chronic cholangitis. After cholecystectomy, these associated symptoms such as fever and abdominal pain relieved completely. Histological examination of gallbladder and the gallstones after surgery confirmed these clinical suspicions.

In summary, this study demonstrates that PKD-induced hemolysis in conjunction with splenectomy may cause severe iron overload even in the absence of *HFE*-genotype abnormalities. The secondary iron overload may lead to chronic liver disease and cirrhosis. Unfortunately, a sufficient causal therapy for PKD is at present not available, although new therapeutic strategies are under evaluation. The most promising gene therapeutic approaches may be either transplantation of heterologous hematopoietic stem cells<sup>[17]</sup> or the reinfusion of autologous stem cells after correction

of the PK gene sequence in the stem cell DNA *in vitro*<sup>[18]</sup>. Until such therapies become available, we suggest a close monitoring of the iron metabolism of PKD patients and a consequent treatment of iron overload.

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