Ferroportin-related haemochromatosis associated with novel Y64H mutation of the *SCL40A1* gene

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Abstract

In this paper we described the first Polish patient with ferroportin disease. Hereditary haemochromatosis (HH) is a condition associated with universal iron overload, and it is divided into four types, according to the Online Mendelian Inheritance in Man (OMIM) database. Ferroportin disease represented a rare type of HH, with autosomal dominant trait of inheritance. In our patient we detected a novel mutation in the ferroportin gene, with non-classical phenotype.

Introduction

Haemochromatosis (HH) is a well-defined condition characterised by normal iron-driven erythropoiesis and the toxic accumulation of iron in parenchymal cells of the liver, heart, and endocrine glands. It can be caused by mutations that affect any of the proteins that limit the entry of iron into the blood [1]. The most common form of HH is associated with HFE gene mutation and called HFE-hemochromatosis. The other form of HH are named non-HFE. Non-HFE haemochromatosis syndromes define a subgroup of hereditary iron loading disorders that share the autosomal recessive trait, the pathogenic basis (i.e. lack of hepcidin synthesis or activity), and key clinical features with classic HFE-haemochromatosis. They are caused by pathogenic mutations in other genes, such as transferrin receptor 2 (*TFR2*), hepcidin (*HAMP*), haemojuvelin (*HJV*), and ferroportin (*FPN*), and, unlike *HFE*-haemochromatosis, they are not restricted to Caucasians. Ferroportin disease, the most common non-HFE hereditary iron-loading disorder, is caused by a loss of iron export function of ferroportin (FPN) resulting in early and preferential iron accumulation in Kupffer cells and macrophages with high ferritin levels and low-to-normal transferrin saturation. This autosomal dominant disorder usually has milder expressivity than haemochromatosis [2].

We report a 53-year-old patient with a hepcidin-resistant form of hereditary haemochromatosis (HH) – ferroportin disease. Unlike the most common HFE-related form of hereditary haemochromatosis, this condition has an autosomal dominant trait of transmission. Our patient was found to be a carrier of a novel Y64H mutation in exon 3 of the *SCL40A1* gene. In the described subject the disease was associated with a non-classical phenotype manifesting itself with more severe hepatic iron overload and more advanced liver fibrosis.

Case report

A 53-year-old male patient was admitted to the Liver Unit of the Pomeranian Medical University with clinical suspicion of liver cirrhosis. He complained of generalized weakness, fatigue, and recurrent epigastric pain. Physical examination revealed no abnormalities. His blood analysis showed hypercholesterolaemia and impaired glucose tolerance. Liver biochemistry showed mild hypertransaminasaemia with alanine transaminase (ALT) > aspartate aminotransferase (AST) and slight elevation of alkaline phosphatase (ALP). Other laboratory tests demonstrated high ferritin of > 6000 ng/dl (refer-

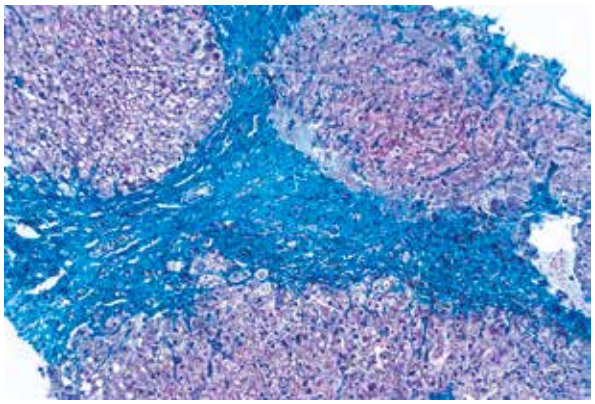

Figure 1. Liver biopsy (dyeing aniline blue, 100×)

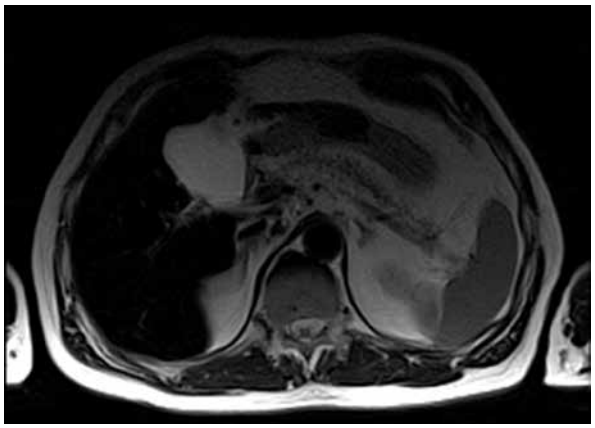

Figure 2. Magnetic resonance imaging scan of the iron overloaded liver

ence value 28–365 ng/ml) and high transferrin saturation of up to 98%, with normal iron of 150 μg/dl (33–193 μg/dl) and low UIBC. H63D and C282Y *HFE* gene mutations were excluded. Liver biopsy revealed features of advanced fibrosis fulfilling histological criteria of cirrhosis and deposits of haemosiderin in hepatocytes, macrophages, and ductal epithelium (Figure 1). Despite putting the patient on a regular phlebotomy protocol his transferrin saturation and ferritin levels did not show significant improvement over the period of 2 years with marked iron overload of parenchymal organs on magnetic resonance imaging (MRI) scans (Figure 2). However, the patient has remained clinically well with Child-Pugh score A and no episodes of decompensation of his liver function. Mutations of the *TfR2* gene were subsequently excluded, and lastly the Y64H mutation of the ferroportin gene was found – a heterozygote exon 3 ferroportin mutation c.190T>C (p. Y64H or Tyr64His).

Discussion

Haemochromatosis is a hereditary iron overload disorder resulting from dysregulation of iron homeostasis [1]. The disease is usually transmitted as an autosomal recessive trait and is mainly due to mutations of the *HFE* gene. The minority of cases are associated with *TfR2* gene mutations and severe, early onset forms are associated with mutations of the haemojuvelin (HJV) and hepcidin (HAMP) genes. More recently, an autosomal dominant form of hereditary haemochromatosis (HH) has been described (ferroportin disease) due to mutations in the ferroportin 1 gene (also known as solute carrier family 40, member A1, or SCL40A1) [3].

The ferroportin gene, SCL40A1, encodes a 62.5 kDa protein which localises to the cell surface and is postulated to contain 12 transmembrane domains. Ferroportin is the only known iron efflux transporter and is regulated by hepcidin, the key regulator of iron homeostasis. Long-term iron loading resulting from ferroportin disease has a broad spectrum of outcomes depending on the disease-causing mutations. Liver damage, including fibrosis and/or cirrhosis, can occur. The clinical presentation and natural course of ferroportin disease has been documented in individual case reports and small case series [3]. A recent meta-analysis on individuals with *SLC40A1* gene mutations showed that, in contrast to HFE haemochromatosis, ferroportin disease has a high penetrance, is genetically heterogeneous, and is rarely associated with fibrosis [4]. Hyperferritinaemia, a normal to low transferrin saturation, and Kupffer cell iron storage, presenting as hepatic and spleen iron overload, are considered characteristic features of classical ferroportin disease. Increased transferrin saturation and hepatocellular iron overload, in addition to hyperferritinaemia and macrophage iron loading, are both considered characteristic for the non-classical phenotype [3].

A genotype-phenotype correlation was suggested to explain the heterogeneity of ferroportin disease where most mutations (e.g. A77D, D157G, V162del, N174I, Q182H, Q248H, and G323V) are associated with classical ferroportin disease. In this classical form, macrophage iron overload results from cellular iron export deficiency, i.e. loss of ferroportin function. Distinct mutations (e.g. N144H, Y64N, C326Y/S, S338R, and Y501C) have been found in patients who presented with the non-classical phenotype associated with a higher risk of fibrosis and a more severe overload of hepatic iron, as in the case mentioned above [4, 5].

Rivard *et al*., in 2003, reported a Y64N mutation-associated ferroportin disease in a large pedigree [6]. However, this amino acid change would imply a T>A nucleotide change at the cDNA level, and not the T>C change described in their report [6]. In fact, the T>C nucleotide change at position 190 of the cDNA (codon TAC, coding for tyrosine 64, Y) results in CAC, coding for histidine, H, not asparagine, N. In the Human Gene

Mutation Database (http://www.hgmd.org/) the ferroportin mutation described by Rivard *et al*. is reported as a T>A change (Y64N).

The *FNP* gene mutation in our case has a different AA change, but of the same amino acid position described in the French-Canadian family [6]. The mutation is located in the first transmembrane domain of the ferroportin 1 protein, suggesting that this part of the ferroportin protein may act as a functional binding site for proteins, such as apotransferrin, ceruloplasmin, or hephaestin, which is important for the export of iron from the cell [6]. In a number of *in vitro* studies it has been shown that the Y64N mutation is associated with resistance to hepcidin regulation. Hepcidin is the main circulating inhibitor of iron flux from enterocytes, hepatocytes, macrophages, and placental cells into the bloodstream, producing this effect by binding to ferroportin. Hepcidin, secreted by hepatocytes into the circulation in response to pro-inflammatory cytokines, hepatocellular iron loading, and endoplasmic reticulum stress, directly interacts with ferroportin and induces its internalisation and degradation. Ferroportin inactivation is mediated by the hepcidin, and lack of binding site for this protein, due to Y64H mutation, may result in intracellular retention of the iron export pump, and non-classical form of the disease. In fact, our patient presented with increased transferrin saturation, massive parenchymal iron overload in the liver, with iron-spared Kupffer cells, and no iron accumulation in the spleen, as documented by MRI – a phenotypic expressivity identical to classic haemochromatosis. Therefore, Y64H mutation of the *FPN* gene belongs to haemochromatosis resistant to hepcidin, and this report is the first Polish ferroportin-related haemochromatosis case of non-classical phenotype.

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