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Severe iron overload with a novel aminolevulinate synthase mutation and hepatitis C infection. A case report

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ABSTRACT

A 55 year old man with a history of chronic hepatitis C infection was found to have severe hemochromatosis: hepatic cirrhosis, cardiomyopathy, arrhythmia, hypogonadism, diabetes and bronzed skin color. After 50 phlebotomies, he underwent a combined heart and liver transplant. Genetic analyses identified a novel mutation in the iron responsive element of the *ALAS2* gene. No mutations were found in other genes associated with adult or juvenile hemochromatosis including *HFE*, transferrin receptor-2 (*TFR2*), ferroportin (*SLC40A1*), hepcidin (*HAMP*) and hemojuvelin (*HJV*). We suggest that the *ALAS2* mutation together with chronic hepatitis C infection may have caused the severe iron overload phenotype.

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Introduction

Erythroid-specific δ-aminolevulinate synthase 2 (*ALAS2*) is the first and rate-limiting enzyme in the heme biosynthetic pathway. Coding region and promoter mutations in *ALAS2*, an X-linked gene, often cause defective or diminished enzyme activity leading to reduced protoporphyrin IX synthesis, accumulation of iron, and insufficient hemoglobin levels [1,2]. The low blood hemoglobin level drives an increase in erythropoiesis causing increased iron uptake and tissue loading. Clinically, patients present with moderate to severe sideroblastic anemia, and hemochromatosis from an early age disproportionate with the number of transfusions received. Males with hemizygous *ALAS2* mutations are often more severely affected but variable penetrance has been reported between brothers with identical mutations [3]. Women heterozygous for *ALAS2* mutations often manifest milder disease, but cases with severe disease have been observed and attributed to skewed X-inactivation [4,5].

ALAS2 is regulated at the translational level by the presence of an iron responsive element (IRE) located in the 5′ untranslated region (UTR) of the mRNA [6]. Under low iron conditions, IRE binding proteins (IRPs) bind to 5′ IREs and block translation. Under high iron conditions, iron is bound to the IRPs; the FeS-IRP is then unable to bind to the 5′ IRE and translation proceeds normally.

Mutations in 5′ IREs have been associated with disease resulting from overexpression of the unregulated gene. Examples include mutations in the 5′ IRE of ferritin light chain, associated with autosomal dominant hereditary hyperferritinemia cataract syndrome [7], and mutation in the 5′ IRE of ferritin heavy chain, associated with autosomal dominant hyperferritinemia [8]. Deletion of the 5′ IRE in ferroportin in the *Pcm* mutant mouse increases intestinal and hepatic expression of ferroportin, leading to reticuloendothelial iron overload [9].

Modest iron overload has been observed in subjects with chronic hepatitis C infection and end-stage liver disease [10]. Hepatitis C infection should not, in and of itself, lead to severe iron overload of the heart and liver, hypogonadism, diabetes and bronzing of the skin. The association of mutations in the gene responsible for the most common form of primary hemochromatosis, *HFE*, with an increase in the severity of hepatic iron overload and end-stage liver disease in patients with hepatitis C infection is controversial at best [11,12]. Nevertheless, *HFE* hemochromatosis patients with hepatitis C infection generally do not exhibit cardiac iron loading and hypogonadism. In contrast, primary hemochromatosis associated with severe liver and heart iron loading, cardiomyopathy and hypogonadism are more often associated with mutations in juvenile hemochromatosis genes, hemojuvelin (*HJV*) [13], and hepcidin (*HAMP*) [14].

In this report, we describe a patient with chronic active hepatitis C infection, severe liver and cardiac iron overload, hypogonadism, diabetes and bronzed pigmentation. Sequence analyses determined that he was hemizygous for a novel mutation in the 5′ IRE of the *ALAS2* gene, and did not have mutations in any known genes associated with

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primary hemochromatosis: *HFE*, *TFR2*, *SLC40A1*, *HJV* and *HAMP*. This case suggests that mutations in the IRE of *ALAS2* can be associated with iron overload disease, especially if additional risk factors such as hepatitis C are present.

Methods

Human subjects

The performance of this work was approved by the Internal Review Board for the use of Human Subjects Committee of The Scripps Research Institute. All samples described in this study were obtained with informed consent.

Genetic screening

Direct sequencing of the genes for ALAS2, *HFE*, *TFR2*, *SLC40A1*, *HAMP* and *HJV* were performed as previously described [15–18].

Iron responsive element gel retardation assays

K562 cells were treated overnight with deferral (100 μM) and lysates prepared and used in gel retardation assays. RNA probes were made by transcription with oligos for ALAS2 wildtype: CTTGAACC-TAAAGTCCTGTTGCCCTGCACTGAG**G**ACGAACGAATGACAGGTGCGCCC-TATAGTGAGTCGTATTA or ALAS2 mut: CTTGAACCTAAAGTCCTGTTG CCCTGCACTGAG**A**ACGAACGAATGACAGGTGCGCCCTATAGTGAGTCG-TATTA, ^{32}P UTP, T7 primer and T7 RNA polymerase. Gel retardation was performed in a 20 μl reaction mix containing the RNA IRE ^{32}P probe (0.1 pmol), 200 μg K562 cell lysate, 10 mM Hepes pH 7.4, 40 mM KCl, 3 mM MgCl2, 5% glycerol, 12.5 mM dithiothreitol, 1U RNasin, and the non-radioactive competitor as indicated. Incubation was performed on ice for 15 min and at room temperature for 15 min followed by electrophoresis on a 5% polyacrylamide/0.5× TBE gel.

Case report

The patient, born in 1950, was found to have hepatitis C in 1980. Brief treatment with interferon and ribavirin in the 1990s was poorly tolerated. In 2003, liver biopsy showed advanced liver disease, and studies revealed massive esophageal varices and moderate splenomegaly. Ascites and encephalopathy emerged in 2004, as did paroxysmal atrial fibrillation. At that time there was "dark bronzed" skin, jaundice, gynecomastia and testicular atrophy. Transferrin saturation was 97%, serum ferritin >1500 ng/ml, and MRI scan by T2* method showed severe iron deposition in liver (7 ms; normal >19) and heart (10 ms; normal >20) (Fig. 1). EKG showed left anterior fascicular block, poor R wave progression and long QT interval. On echocardiogram and cardiac catherization, there was global hypokinesis, ejection fraction 21–30%, and normal coronary arteries. Endocrine studies showed hypogonadotropic hypogonadism (LH 1.2, testosterone 140, free testosterone 19.5, all below normal), adrenal insufficiency (AM cortisol <0.1 and PM 7 μg/dL, ACTH 17 pg/mL) and normal thyroid function. Oral hypoglycemics were started for early diabetes mellitus. Hemoglobin was 13.3 g/dL, hematocrit 37%, MCV 117, WBC 8100, platelets 61×10^9/L. Phlebotomy therapy was initiated

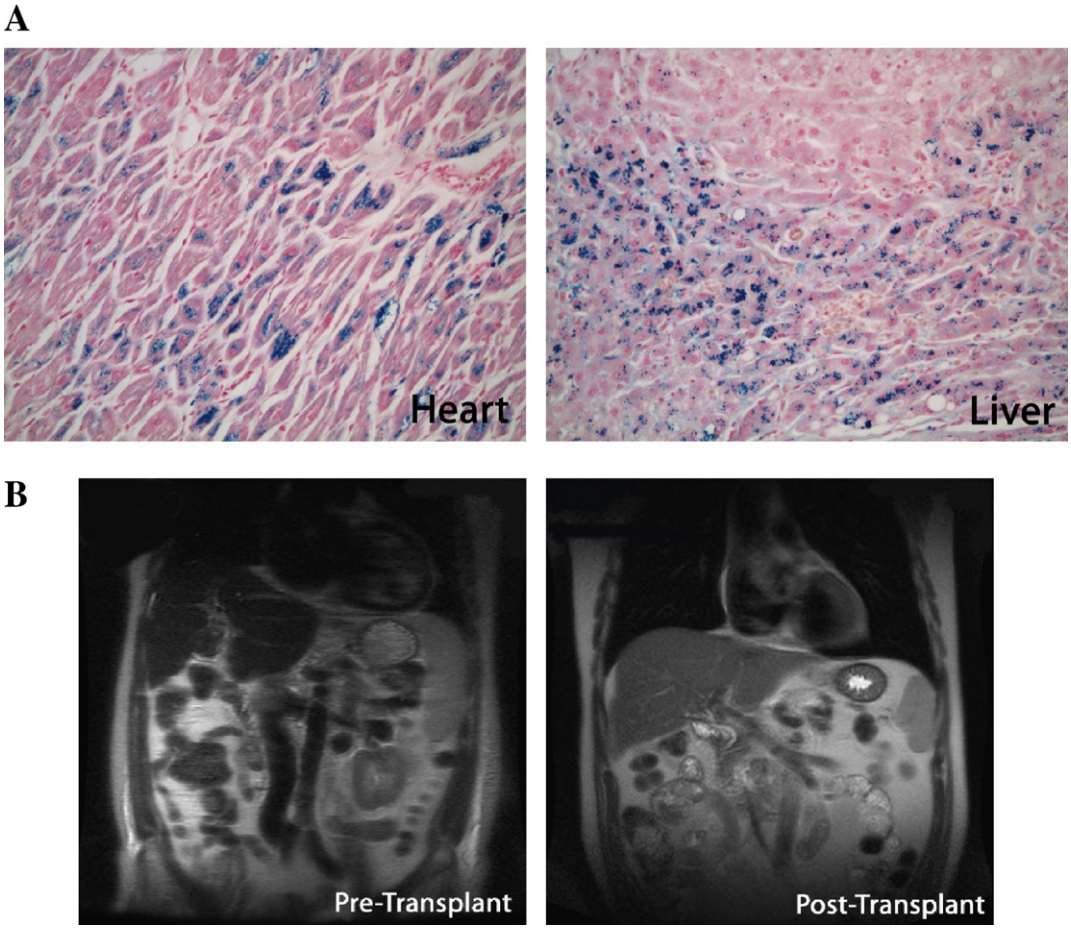

Fig. 1. (A) Histology of the heart and liver showing severe iron loading (20× magnification; Prussian blue). (B) MRI scans before and after transplantation. The markedly attenuated T2-weighted signal intensity of heart and liver revert to normal post-transplant.