

## Is serum hepcidin causative in hemochromatosis? Novel analysis from a liver transplant with hemochromatosis

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PC Adams, V McAlister, S Chakrabarti, M Levstik, P Marotta. Is serum hepcidin causative in hemochromatosis? Novel analysis from a liver transplant with hemochromatosis. *Can J Gastroenterol* 2008;22(10):851-853.

**BACKGROUND:** Heparin is a circulating hepatic hormone that regulates iron balance. It has been speculated that hepcidin insufficiency or dysregulation may be the primary defect in genetic hemochromatosis.

**METHODS:** A 62-year-old woman underwent elective liver transplantation for chronic hepatitis C cirrhosis. Genetic testing for hemochromatosis was subsequently performed on the donor and recipient. Liver iron concentration was measured in the donated liver at the time of transplantation, and at day 2 and day 652 post-transplant. Serum hepcidin was measured at day 935 in the recipient and in three other liver transplant recipients.

**RESULTS:** The donor was discovered to have significant iron overload without fibrosis, with a liver iron concentration of 326 µmol/g (normal is 0 µmol/g to 35 µmol/g). Genetic testing confirmed that the 89-year-old female donor was a typical C282Y homozygote for hemochromatosis. The recipient did not carry either the C282Y or the H63D mutation of the *HFE* gene for hemochromatosis. Liver biopsy was performed on the recipient on day 2 and day 652 post-transplant; the liver iron concentrations were 333 µmol/g and 253 µmol/g, respectively. Serum hepcidin in the recipient was elevated at 111 ng/mL compared with that of the three other ambulatory liver transplant recipients (66 ng/mL, 76 ng/mL and 81 ng/mL).

**CONCLUSION:** The liver transplant recipient described in the present report demonstrated a slight decrease in liver iron concentration over a 1.8-year follow-up period without specific therapy. Heparin insufficiency as a primary cause of genetic hemochromatosis seems unlikely based on the clinical profile of the present patient and the hepcidin measurements.

**Key Words:** Hemochromatosis; HFE; Iron overload

The pathogenesis of genetic hemochromatosis remains elusive despite the discovery of the *HFE* gene in 1996 (1). Several investigators have suggested that serum hepcidin – a hepatic hormone that controls iron balance (2) – may be the primary abnormality in patients with genetic hemochromatosis. An inappropriately low hepcidin level has been demonstrated

### L'hepcidine sérique provoque-t-elle une hémochromatose ? Une analyse novatrice d'une greffe du foie avec hémochromatose hépatique

**HISTORIQUE :** L'hepcidine est une hormone hépatique circulante qui régule l'équilibre du fer. On avance qu'une carence ou une dysrégulation de l'hepcidine pourrait être la principale anomalie responsable de l'hémochromatose génétique.

**MÉTHODOLOGIE :** Une femme de 62 ans a subi une greffe hépatique non urgente à cause d'une cirrhose secondaire à l'hépatite C. Des tests génétiques d'hémochromatose ont ensuite été effectués sur la donneuse et la receveuse. On a mesuré la concentration de fer dans le foie greffé au moment de la greffe, puis le deuxième jour et le 652<sup>e</sup> jour après la greffe. On a mesuré l'hepcidine sérique le 935<sup>e</sup> jour chez la receveuse et chez trois autres greffés du foie.

**RÉSULTATS :** On a découvert que la donneuse présentait une importante surcharge de fer sans fibrose, la concentration de fer dans le foie s'élevant à 326 µmol/g (normale de 0 µmol/g à 35 µmol/g). Les tests génétiques ont confirmé que la donneuse de 89 ans était une homozygote C282Y classique à l'hémochromatose. La receveuse n'était porteuse ni de la mutation C282Y ni de la mutation H63D du gène *HFE* de l'hémochromatose. La receveuse a subi une biopsie du foie le deuxième jour et le 652<sup>e</sup> jour après la greffe, et les concentrations de fer dans le foie s'élevaient alors à 333 µmol/g et à 253 µmol/g, respectivement. L'hepcidine sérique de la receveuse était élevée, à 111 ng/ml, par rapport à celle de trois autres greffés du foie ambulatoires (66 ng/ml, 76 ng/ml et 81 ng/ml, respectivement).

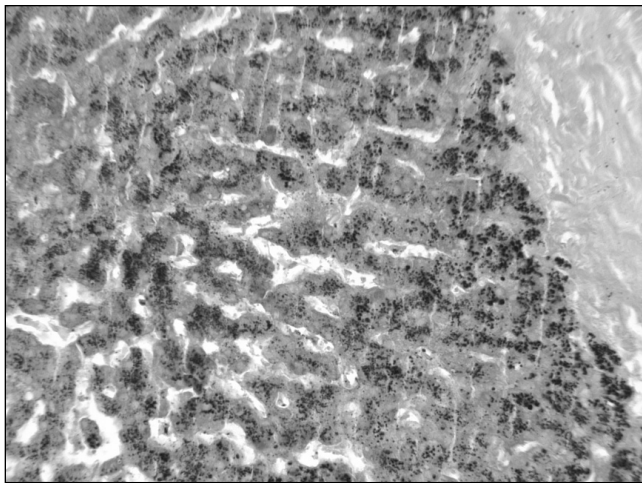
**CONCLUSIONS :** La greffée du foie décrite dans le présent rapport démontre une légère diminution de la concentration de fer dans le foie pendant un suivi de 1,8 an sans thérapie spécifique. L'insuffisance en hepcidine comme cause principale d'hémochromatose génétique semble peu probable d'après le profil clinique de la présente patiente et les mesures d'hepcidine.

in serum from hemochromatosis patients. A low serum hepcidin leads to an increase in intestinal iron absorption and subsequent hepatic deposition of dietary iron (3-6). There have been previous reports of liver transplantation occurring from a C282Y homozygous donor into a recipient (7) and transplantation of a normal liver into a C282Y homozygous recipient.

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Received for publication January 15, 2008. Accepted April 11, 2008



**Figure 1** Liver biopsy at the time of liver transplantation demonstrated a liver iron concentration of 326  $\mu\text{mol/g}$  (reference range 0  $\mu\text{mol/g}$  to 35  $\mu\text{mol/g}$ ; Perl's stain for iron)

Iron reaccumulation has been an inconsistent observation, although the follow-up time may not have been adequate (8,9). The simultaneous transplantation of the liver and small intestine from a C282Y homozygous donor has led to iron overload in the recipient within two years (10). The case described in the present report provides a clinical opportunity to observe the effects of liver transplantation of a C282Y homozygous liver into an unaffected recipient without phlebotomy treatment, and without anemia or clinical bleeding. The development of a new assay for serum hepcidin provides new information on the role of hepcidin in the pathogenesis of hemochromatosis.

### METHODS

Liver iron absorption was measured by atomic absorption spectrophotometry. Genetic testing for the C282Y and H63D mutation of the *HFE* gene was done from a buffy coat prep on the liver donor and recipient, as previously described (11). Serum hepcidin was measured using an enzyme-linked assay, utilizing a polyclonal antibody developed by Dr Tomas Ganz (Intrinsic LifeSciences, USA) (12). The assay is available for research purposes only, and it has been estimated that a typical serum hepcidin level is 50 ng/mL in women and 100 ng/mL in men.

### CASE PRESENTATION

A 62-year-old woman underwent an elective liver transplantation for chronic hepatitis C cirrhosis in May 2005. She had been successfully treated for hepatitis C before the liver transplantation and was HCV RNA-negative. The explanted liver demonstrated no evidence of iron overload. The donor was an 89-year-old woman who had a cerebral aneurysm and no clinical evidence of liver disease. At the time of donor liver retrieval, an analysis of a frozen section demonstrated iron overload (Figure 1). Post-transplantation review of the donor biopsy confirmed iron overload with a liver iron concentration of 326  $\mu\text{mol/g}$  (normal range 0  $\mu\text{mol/g}$  to 35  $\mu\text{mol/g}$ ). The donor was confirmed to carry two copies of the C282Y mutation of the *HFE* gene (C282Y homozygote) and the recipient carried neither the C282Y nor the H63D mutation (wild-type). Transplant surgery was complicated by hepatic artery stenosis and dissection. Therefore, on day 2,

intraoperative repair was performed. A liver biopsy was also performed, which demonstrated a liver iron concentration of 333  $\mu\text{mol/g}$ . The subsequent course of the patient as an out-patient was dominated by multiple endoscopic retrograde cholangiopancreatography examinations to dilate a persistent biliary anastomotic stricture, which was likely secondary to early hepatic ischemia. At day 652, a liver biopsy was performed because of a new pattern of elevated liver enzymes. The liver iron concentration was measured at 253  $\mu\text{mol/g}$  with no fibrosis. At day 935 post-transplant, the patient was noted to have a serum ferritin level of 4999  $\mu\text{g/L}$  (reference range 15  $\mu\text{g/L}$  to 200  $\mu\text{g/L}$ ) and a transferrin saturation of 100% (reference range, 20% to 55%). Other blood test results at day 935 included the following: hemoglobin 149 g/L, aspartate aminotransferase 30 U/L, alanine aminotransferase 34 U/L, alkaline phosphatase 53 U/L, erythrocyte sedimentation rate 1 mm/h and C-reactive protein less than 3 mg/L. Serum hepcidin in the transplant recipient at day 940 was 111 ng/mL. Serum hepcidin was measured in three other ambulatory liver transplant patients without iron overload and was 66 ng/mL, 76 ng/mL and 81 ng/mL. The patient has begun a program of 500 mL phlebotomy every two weeks to decrease liver iron.

### DISCUSSION

In the present case, it is intriguing to speculate on whether we have transplanted the genetic defect of hemochromatosis into the recipient. An *HFE* knockout mouse specific for hepatocytes has been demonstrated to develop iron overload (13). In the present patient, the absence of progression of iron overload over 1.8 years goes against this hypothesis. A novel aspect of the present case is the ability to measure serum hepcidin. It has been proposed that hepcidin and iron are analogous to insulin and glucose as regulatory hormones and substrates (4). In this hypothesis, patients with typical hemochromatosis and some other forms of iron overload have a low and inappropriate serum hepcidin level, which leads to increased intestinal iron absorption. In the present case, our patient had the highest serum hepcidin of the four liver transplant recipients that were tested. An elevated serum hepcidin would be expected to decrease iron absorption, which is consistent with the absence of iron accumulation in our patient. It is possible that liver transplantation, rejection and inflammation could elevate hepcidin, but presumably this would apply to all of the transplant patients, and our patient had a low erythrocyte sedimentation rate and a low C-reactive protein level, without significant inflammation on liver biopsies. If the recipient had received the primary defect of hemochromatosis as a result of the transplanted liver, she would be expected to have a low serum hepcidin and progressive iron overload over time. The present report raises the possibility that other factors control the regulation of hepcidin in hemochromatosis, and extrahepatic production of hepcidin may be another explanation. We have previously reported progressive iron overload after the simultaneous transplantation of the liver and intestine from a C282Y homozygous donor (7), suggesting that there may be a signal from the hemochromatosis intestine that affects hepcidin and iron absorption.

### CONCLUSIONS

Based on the present case, it seems unlikely that hepcidin insufficiency is the primary genetic defect of C282Y-linked hemochromatosis.

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