CASE REPORT

Occult celiac disease prevents penetrance of hemochromatosis

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INTRODUCTION

Hereditary hemochromatosis (HH) is an inherited disorder of iron metabolism that is characterized by excessive gastrointestinal iron absorption with subsequent iron deposition in major organs of the body[1,2]. Autosomal recessive transmission of the HH gene and genetic linkage to the HLA complex has been known since the mid-1970s[3,4]. In 1996 a candidate gene for HH, now known as HFE, was detected by positional cloning[5]. Two missense mutations were initially identified in the HFE gene, leading to an exchange of cysteine to tyrosine at position 282 (C282Y) or a change from histidine to aspartate at position 63 (H63D). Although the homozygous C282Y genotype affects between 1 in 200 and 1 in 400 persons of northern European descent, only a considerably low percentage of these homozygotes develop clinically severe hemochromatosis[6]. Thus, genetic or environmental factors affecting the penetrance of hemochromatosis in patients with HFE mutations still remain to be elucidated.

Iron deficiency anemia in patients with HH is very rarely discovered due to proposed algorithms, which suggest genotyping only in patients with fasting transferrin saturation higher than 45%[1-3]. However, rare cases are documented and may be associated with disorders leading to duodenal atrophy with subsequent iron malabsorption. To date only three patients with HH and iron deficiency anemia associated with celiac disease (CD) have been reported in the world literature[7-9]. In this contribution, we report another patient with C282Y homozygosity, depleted body iron and intestinal atrophy caused by CD. Whereas in HH patients without CD divalent-metal transporter 1 (DMT1) expression is up-regulated leading to excessive uptake of iron, we identified a significant reduction in protein and mRNA expression of DMT1 as a compensatory mechanism in this patient with HH and CD.

CASE REPORT

A 65-year-old Caucasian female patient presented with chronic diarrhea and weight loss of 10 kg over 4 years. Diarrheal episodes occurred in association with cereals and milk products. An initial lactose-free diet failed to improve symptoms but a recent trial of gluten-free diet brought partial relief at the time of referral. Her history was unremarkable except for the fact that she suffered from hemochromatosis 14 years ago and was treated with phlebotomy over nearly a decade. Two years ago HFE genotyping detected a homozygous C282Y mutation. Nevertheless an oral iron substitution therapy had to be started because she developed an iron deficiency anemia. The initial evaluation revealed...
the persistence of iron deficiency anemia (Table 1). Endomysium (1:160, reference <1:110), gladin (IgG 37 kU/L, IgA 740 kU/L, reference <12 kU/L each) and tissue transglutaminase antibodies (253 U, reference <20 U) were strongly positive. Histological examination of small bowel biopsies detected intestinal atrophy with lymphocytic infiltrates suggestive for CD. Within 3 mo of gluten-free diet, diarrhea completely resolved and milk products were tolerated well. Iron substitution was stopped when serum iron, ferritin and hemoglobin reached normal values after 4 mo of gluten-free diet (Table 1). She gained 15 kg of weight within 1 year. Control small bowel biopsies showed resolution of the enteropathy, and a breath test with H₂ lactose proved resolution of the secondary lactose intolerance.

Table 1 Clinical chemistry data

|                      | First visit time of biopsy | End of follow up control biopsy | Reference values |
|----------------------|----------------------------|--------------------------------|------------------|
| Hemoglobin (g/L)     | 112                        | 153                            | 120-160          |
| Hematocrit           | 35                         | 45                             | 0.37-0.47        |
| Serum iron (μmol/L)  | 7.0                        | 26.0                           | 11.1-31.1        |
| Transferrin (g/L)    | 2.5                        | 1.9                            | 2.0-3.6          |
| Transferrin saturation (%) | <15                     | 54                             | 16-45            |
| Ferritin (µg/L)      | 11                         | 78                             | 30-150           |

Under iron substitution 100 mg q.d.

Assays

Serum clinical chemistry was performed by standard techniques with an autoanalyzer.

HFE genotyping

Genomic DNA was extracted from whole blood and light cycle (Roche, Mannheim, Germany) amplification of the H63D and C282Y variant fragments with subsequent melting curve analysis was performed using standard cycle conditions. The C187G transition at codon 282 was monitored with the 3'-fluorescein-labeled sensor 5'-AGATATACGTAC-TCATCATAGAACACGAACA-3' probe. The G845A transition at codon 282 was simultaneously analyzed with the 3'-fluorescein-labeled sensor 5'-AGATATACGTAC-CAGGGTGAG-3' and the 5'-LC-Red640-labeled anchor probe 5'-CCAGGGCTGGATACAGCCCTACTGTGAGCAAGC-3'. The inverse regulation of basolateral FP1 mRNA was only slightly increased during follow-up. Consistent with our immunohistochemistry, TfR1 expression drastically decreased in line with regeneration after therapy (immunohistochemistry not shown).

To quantify these changes in expression during treatment mRNA levels of DMT1, FP1, and TfR1 were determined by real-time PCR. DMT1 mRNA expression was minimal with substantial atrophy of the duodenal epithelium but was elevated fourfold after therapy. In contrast, TfR1 expression was inversely related to body iron stores and fell to one-third with regeneration of the epithelium and restoration of iron uptake (Figure 2). Consistent with our immunohistochemistry, FP1 mRNA was only slightly increased during follow-up. These findings indicate that the inverse regulation of basolateral TfR1 expression which is believed to be a sensor for the body's need for iron, was functional in our patient with HH, whereas DMT1 appeared to be differentially regulated in comparison to other patients with hemochromatosis but no CD.

DISCUSSION

Over 90% of patients with HH are homozygous for the C282Y mutation of the HFE gene, but the majority of patients,
who have been identified as being homozygous for the C282Y mutation, have no evidence of iron overload. The best current estimate of penetrance for C282Y homozygosity is that less than 1% of homozygotes develop frank clinical hemochromatosis\cite{6}. In contrast, another population based study found a higher prevalence of 50%\cite{11}, but these data are difficult to interpret since clinical findings are not matched to a control group. Overall population prevalence for clinical HH and HH-related death appears to be far less than expected from C282Y allele frequency and heterozygosity frequency\cite{12}.

Normal HFE protein is expressed in cryptal enterocytes of the small intestine and acts to facilitate the iron-sensing function of these cells by a physical association with the transferrin receptor (TfR1) at a strategic site to influence TfR1-mediated iron transport\cite{13}. This facilitory effect is lost by C282Y mutant HFE resulting in a 'relative' iron deficiency of enterocytes and an uncoupling of iron uptake regulation\cite{2,14}. This uncoupling leads to increased expression of the divalent-metal transporter 1 (DMT1) and ferroprotein 1 (FP1)\cite{2,14,17}.

Whereas DMT1 represents a transmembrane transporter in the apical membrane of duodenal enterocytes which facilitates iron uptake from the intestinal lumen into the enterocytes, FP1 is involved in basolateral iron export into the portal circulation\cite{2,14}.

Iron transport across the basolateral plasma membrane of villus enterocytes involves intact epithelia with a sufficient amount of basolateral and apical transport proteins\cite{2,14}. Atrophy of the duodenal mucosa in chronic inflammatory disorders of the small bowel such as CD leads to iron deficiency\cite{15}. Our immunohistochemistry data implicate that a marked reduction of basolateral DMT1 in the atrophic intestinal epithelium causes secondary malabsorption of iron and prevents clinical iron overload. A control biopsy taken on gluten-free diet showed resolved duodenal enteropathy paralleled by ferritin levels within the normal range. Restored iron uptake was accompanied with increased expression of DMT1, which was higher than levels usually present in healthy controls in both immunohistochemical staining and real-time PCR. This pattern of transporter expression after therapy meets with previous data from patients with HH, which show an increased expression of DMT1\cite{10,16,17}. In contrast, FP1 expression was not restored during the observation period but showed a tendency to increase similar to HH patients without CD\cite{10,16,17}. The data clearly demonstrate that the impaired expression of iron transporters in enteropathic mucosa is different from patients with HH and iron overload and compensates for the uncoupling of iron uptake in homozygous C282Y HFE mutants. DMT1 down-regulation in patients with both HH and CD may cause even iron deficiency and anemia although the transferrin receptor is still physiologically regulated by the body’s iron store. Increased duodenal tissue levels of transferrin receptor expression during epithelial atrophy rather favor a selective regulatory

**Figure 1** Immunohistochemical staining for DMT1 and TfR1 of duodenal biopsy specimens of the patient taken before (upper panels A and B) and after gluten free diet (lower panels C and D).
process leading to DMT1 down-regulation than an unselective loss of specialized epithelium. Based on these quantitative data and the clinical recovery of our patient from iron deficiency on a gluten-free diet, we hypothesize that CD masks overt HH due to a reversible reduction in the iron transporter DMT1 and therefore could prevent the penetrance of HH.

CD accounts for up to 8.5% of iron deficiency anemia of unknown etiology, especially when refractory to oral supplementation, and iron deficiency appears to be the most frequent and sometimes only extraintestinal symptom in CD. Recovery from iron deficiency anemia in CD usually occurs between 6 and 12 mo on gluten-free diet alone as a consequence of normalization of histological alterations on the intestinal mucosa. The prevalence of this disease is difficult to ascertain, because many patients have atypical symptoms or none at all, but it seems more common than previously considered and ranges between 0.5% in the general population and 1.6% among patients undergoing endoscopy. CD and hemochromatosis are common HLA-defined conditions with surprisingly high frequencies in populations of Northern Europe commonly attributed to survival advantages. A genetic association between CD and the HLA-D locus has emerged and it has been shown that over 95% of patients express the DQa1*0501 DQB1*0201 heterodimer (HLA DQ2). This locus on chromosome 6p is in close proximity to the HFE locus. Recently, HFE gene mutations have been found to be common and in linkage disequilibrium with different HLA alleles in CD patients compared with controls. A disease specific haplotype that carries both the C282Y HFE gene mutation and HLA DQ2 has been suggested but the origins of the genetic linkage still remain to be investigated in detail. The HFE gene may have spread due to the protection of heterozygotes against iron deficiency and the same might be true for CD which diminishes iron overload. We hypothesize that the genetic predisposition for either disease ameliorates the manifestation of the other thereby leading to a marked extent of unidentified disease. Occult CD may prevent increased DMT1 expression in a specific subset of individuals with homozygous C282Y mutations in the HFE gene thus contributing to the low penetrance of HH.

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