CLINICAL ADVANCES IN LIVER, PANCREAS, AND BILIARY TRACT

HFE Genotype, Parenchymal Iron Accumulation, and Liver Fibrosis in Patients With Nonalcoholic Fatty Liver Disease

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This article has an accompanying continuing medical education activity on page e9. Learning Objective: Upon completion of reading this article, successful learners will be able to differentiate the effect of different patterns of iron overload on liver damage, understand the effect of mutations in the *HFE* gene of hereditary hemochromatosis in determining the predisposition to develop iron overload, and recognize the lack of utility of *HFE* mutations assessment in the absence of histological demonstration of hepatocellular iron accumulation in patients with nonalcoholic fatty liver disease.

See related article, Meriden Z et al, on page 289 in *CGH*; see editorial on page 817.

BACKGROUND & AIMS: Mutations in the hemochromatosis gene (*HFE*) (C282Y and H63D) lead to parenchymal iron accumulation, hemochromatosis, and liver damage. We investigated whether these factors also contribute to the progression of fibrosis in patients with nonalcoholic fatty liver disease (NAFLD). **METHODS:** We studied clinical, histologic (liver biopsy samples for hepatocellular iron accumulation), serologic (iron and enzyme levels), and genetic (*HFE* genotype) data from 587 patients from Italy with NAFLD and 184 control subjects. **RESULTS:** Iron accumulation predominantly in hepatocyes was associated with a 1.7-fold higher risk of a fibrosis stage greater than 1 (95% confidence interval [CI]: 1.2–2.3), compared with the absence of siderosis (after adjustment for age, body mass index, glucose tolerance status, and alanine aminotransferase level). Nonparenchymal/mixed siderosis was not associated with moderate/severe fibrosis (odds ratio, 0.72; 95% CI: 0.50–1.01). Hepatocellular siderosis was more prevalent in patients with *HFE* mutations than in those without; approximately one third of patients with *HFE* mutations had parenchymal iron accumulation (range, 29.8%–35.7%, depending on *HFE* genotype). Predominantly hepatocellular iron accumulation occurred in 52.7% of cases of patients with *HFE* mutations. There was no significant association between either the presence of *HFE* mutations or specific *HFE* genotypes and the severity of liver fibrosis. **CONCLUSIONS: Iron deposition predominantly in hepatocyes is associated with more severe liver damage in patients with NAFLD. However, *HFE* mutations cannot be used to identify patients with hepatocellular iron accumulation.**

Keywords: Hepatic Fibrosis; hfe Gene; Iron Overload; Nonalcoholic Fatty Liver Disease.

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Nonalcoholic fatty liver disease (NAFLD), characterized by hepatic[1] and systemic[2,3] insulin resistance and related to the metabolic syndrome,[4] represents the leading cause of alterations of liver enzymes in Western countries, affecting 20%–34% of the population.[5,6] In patients with severe insulin resistance and associated nonalcoholic steatohepatitis (NASH),[4] NAFLD is a potentially progressive liver disease evolving to cirrhosis and eventually to hepatocarcinoma[7–9] and confers an increased risk of liver-related mortality.[10] Inherited factors

Abbreviations used in this paper: BMI, body mass index; GGT, γ-glutamyltranspeptidase; HFE, *hemochromatosis* gene; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

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play a role in the susceptibility to the metabolic syndrome and NASH,[11] and single nucleotide polymorphisms in genes involved in inflammation, oxidative stress, and fibrogenesis have been associated with the severity of liver damage in NAFLD.[12–14]

Hyperferritinemia is observed in up to one third of NAFLD cases and has been associated with oxidative stress and mild hepatic iron accumulation[15–17] sometimes related to the presence of common mutations of the HFE gene responsible for hereditary hemochromatosis.[16,18] Increased liver iron may directly promote fibrogenesis by inducing oxidative stress and stimulating hepatic stellate cells activation through ferritin release,[19] but increased iron stores have also been shown to promote hepatic insulin resistance in rats fed with a high-fat diet.[20] Moreover, iron depletion improved insulin resistance more than lifestyle changes alone in patients with NAFLD.[17]

The C282Y and H63D mutations of the hemochromatosis gene (*HFE*) responsible for hereditary hemochromatosis represent the leading cause of inherited iron overload in individuals of European ancestry.[21] The mechanism is related to decreased hepcidin release leading to increased iron absorption and parenchymal deposition.[22] In white ethnicity patients with NAFLD, hyperferritinemia has been associated with more advanced liver damage, whereas the relationship between HFE mutations and liver fibrosis is controversial.[18,23–26] Conflicting results are possibly related to several causes: (1) low number of patients considered in individual series, precluding the evaluation of the effect of single genotypes on liver damage; (2) different inclusion criteria; (3) lack of the estimate of the relationship between genotypic data and expression of iron overload; (4) different definition of *HFE* genotypes at risk for iron overload. The aim of this study was to determine the relationship among hepatocellular iron accumulation, HFE mutations, and liver damage in a large series of Italian patients with NAFLD.

Patients and Methods

Patients

We considered 587 out of 680 (86.3%) unrelated white ethnicity patients from Italy with biopsy-proven NAFLD diagnosed between January 1999 and January 2008, whose DNA samples and complete clinical data were available. The cohort included 526 patients submitted to liver biopsy because of persistently abnormal liver enzymes/serum ferritin or a long-lasting history of steatosis associated with severe metabolic abnormalities and 61 severely obese patients who were found to be affected by NAFLD at routine liver biopsy performed during bariatric surgery. Ninety-three patients were excluded because of incomplete clinical data or lack of DNA samples; their clinical characteristics were not significantly different from the total cohort. Other causes of liver disease were previously used for exclusion, including increased alcohol intake (>30/20 g/day for males/females, respectively), as confirmed by at least 1 family member or friend and carboxydesialylated transferrin determination, autoimmune liver diseases, hereditary hemochromatosis (C282Y +/+ subjects), AAT deficiency, Wilson's disease, or viral hepatitis (Figure 1). Part of this group had previously been described.[12] Because of the low penetrance of the C282Y/H63D genotype in the general population,[21] subjects carrying this genotype were considered in this study. Body mass index (BMI) and metabolic parameters, including glucose and lipid levels, ferritin, and liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and γ-glutamyltranspep-

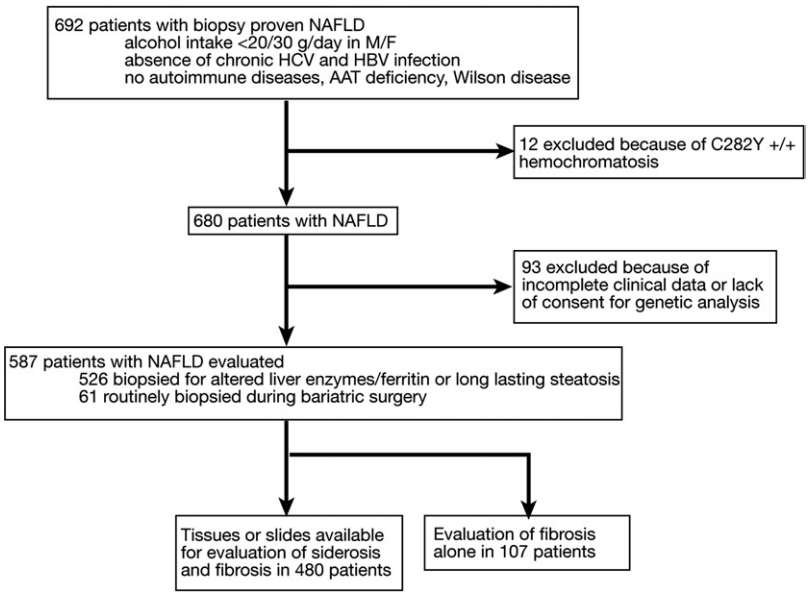

Figure 1. Selection of the study patients.