HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease.

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

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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.

Abbreviations used in this paper

BMI, body mass index;

GGT, γ-glutamyltranspeptidase;

HFE, hemochromatosis gene;

NAFLD, nonalcoholic fatty liver disease;

NASH, nonalcoholic steatohepatitis

Nonalcoholic fatty liver disease (NAFLD), characterized by hepatic1 and systemic2 and 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 and 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 hepatocarcinoma7, 8 and 9 and confers an increased risk of liver-related mortality.10 Inherited factors 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, 13 and 14

Hyperferritinemia is observed in up to one third of NAFLD cases and has been associated with oxidative stress and mild hepatic iron accumulation15, 16 and 17 sometimes related to the presence of common mutations of the HFE gene responsible for hereditary hemochromatosis.16 and 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, 24, 25 and 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-glutamyltranspeptidase [GGT]), were available in all patients. Their demographic and clinical features are shown in Table 1. Subjects lacking the evaluation of histologic iron deposition (n = 107) were more often female, younger, and had a higher BMI because this subgroup included all the patients submitted to bariatric surgery (not shown in detail). The study was approved by the Institutional Review Board of the Ospedale Maggiore Policlinico IRCCS.

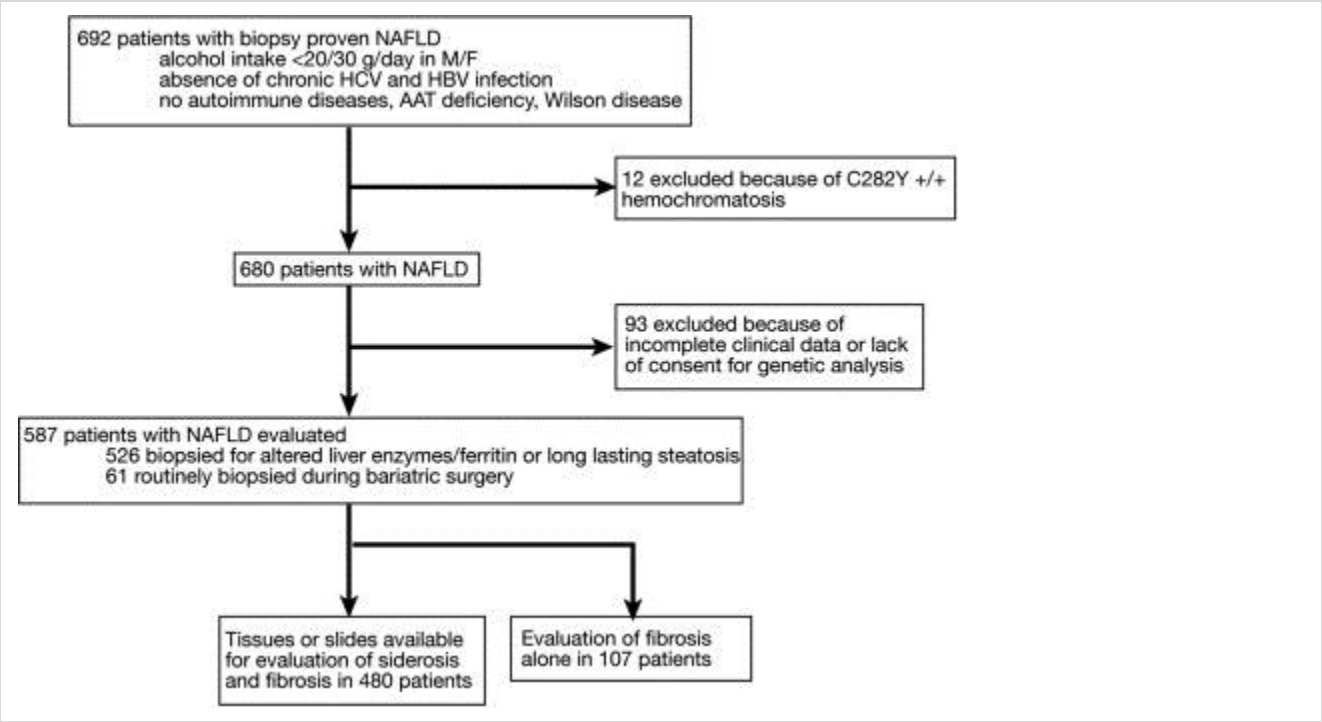

Figure 1.

Selection of the study patients.

Table 1.

Demographic and Clinical Features of 179 Italian Healthy Control Subjects With Normal Liver Enzymes and Metabolic Parameters and 587 Patients With Histologic Diagnosis of NAFLD

	Controls	NAFLD, whole cohort	NAFLD, siderosis available
Number	179	587	480
Sex (*%*)	38 (21)	77 (29)	88 (18)
Age, *y*	48.4 ± 13	45.2 ± 11.6	46.1 ± 11.7
BMI (*kg/m*^{2})	25.1 ± 2.7	29.0 ± 6.2[a]	27.3 ± 3.5[a]
LDL cholesterol, *mg/dL*	118.7 ± 29	128.4 ± 43[a]	129.4 ± 43[a]
HDL cholesterol, *mg/dL*	55.2 ± 13	44.9 ± 12[a]	48.1 ± 13[a]
Triglycerides, *mg/dL*	90.1 ± 44	155.2 ± 84[a]	149.4 ± 88[a]
Glucose, *mg/dL*	89.0 ± 10	98.3 ± 27[a]	98.8 ± 25[a]
HOMA-IR	2.7 ± 1.6	4.3 ± 4.3[a]	4.4 ± 4.5[a]
IGT-IFG/diabetes (*%*)	0/0	80 (14)/64 (11)[a]	72 (15)/46 (10)[a]
ALT, *UI/mL*	21.8 ± 7	66.4 ± 41[a]	67.7 ± 47[a]
GGT, *UI/mL*	23.7 ± 16	87.4 ± 104[a]	90.2 ± 102[a]
Fibrosis stage F0/F1/F2/F3/F4 (*%*)	—	270/166/82/45/24 (46/28/14/8/4)	232/112/69/43/24 (48/23/14/9/5)

NOTE. Of 587 patients with histologic diagnosis of NAFLD, there were 480 available for re-evaluation of histologic siderosis.

HDL, high-density lipoprotein; HOMA-IR, homeostasis models assessment-insulin resistance; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; LDL, low-density lipoprotein.

a

$P < .0001$ between patients and controls.

Controls

The control group included 184 Italian subjects from a larger series of 482 blood donors from Northern Italy without clinical and biochemical evidence of liver and metabolic disease and no alcohol abuse. We excluded subjects with ALT >35/30 IU/mL in males/females, GGT >35 IU/mL, BMI >28, abdominal circumference >100 cm, glucose levels ≥100 mg/dL, triglycerides ≥150 mg/dL, high-density lipoprotein ≤45/55 in males/females, or a fatty liver index >40, a value with high specificity to rule out NAFLD in the general population.27 Informed written consent was obtained from each patient and control subject, and the study conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Histologic Assessment

Tissue sections were stained with H&E, impregnated with silver for reticulin framework, and stained with trichrome for collagen. Two expert pathologists unaware of clinical and genetic data reviewed all biopsy specimens for fibrosis stage and iron accumulation. The presence of NASH was assessed according to Kleiner et al.28 The minimum biopsy sample size was 1.7 cm and the number of portal areas was 10. Histologic re-evaluation of liver siderosis was performed in 480 samples according to Scheuer et al.29 When detected, hepatic iron accumulation was defined as predominantly hepatocellular or nonparenchymal/mixed according to the prevalent distribution pattern of siderosis.

Genetic Analysis

DNA was extracted from peripheral blood by the phenol-chloroform method. Success rate in extracting DNA was 100% for each study group. The HFE genotype was genotyped by restriction analysis by personnel unaware of patients' clinical status. 14 Random samples were confirmed by direct genotyping that provided concordant results in all cases. Samples from both NAFLD patients and controls were included in all batches analyzed, and quality controls were performed to verify the reproducibility of the results. Valid genotypic data were obtained for over 99% of subjects analyzed.

Statistical Analysis

Our sample had a >90% power of detecting an odds ratio (OR) of 1.7 for fibrosis stage >1 in patients with NAFLD for the presence of HFE mutations, with a significance of 5% (2 tailed). Results are expressed as means ± standard deviation or median (interquartile range), when appropriate, and considered significant when $P < .05$ (2 tailed). Mean values were compared by analysis of variance or Wilcoxon test, when appropriate, and frequencies by $\chi 2$ test.

The association between the HFE genotypes and the presence of metabolic abnormalities and fibrosis was evaluated by logistic regression analysis adjusted for confounders (as reported in the Results section). Analyses were carried out with JMP 6.0 statistical analysis software (SAS Institute Inc, Cary, NC).

Results

The frequency distribution of HFE genotypes was not significantly different (P = .6) between patients with NAFLD and controls with normal liver enzymes and metabolic parameters (Table 2). The relationship between HFE genotypes and serum and histologic parameters of iron accumulation is shown in Table 3. In patients with NAFLD, the presence of HFE mutations was associated with significantly higher transferrin saturation levels compared with the wild-type (wt) genotype, and transferrin saturation was higher in patients carrying the C282Y/wt, H63D/H63D, and C282Y/H63D genotypes than in those carrying the H63D/wt genotype (P < .05). Ferritin levels and the prevalence of histologically detectable siderosis, in particular of nonparenchymal siderosis, were also significantly higher in patients carrying the C282Y/wt, H63D/H63D, and C282Y/H63D genotypes than in those carrying the H63D/wt and wt/wt genotypes. Similarly, hepatocellular siderosis was significantly more prevalent in patients carrying HFE mutations than in those who were negative but was expressed in 42.7%–76.5% of HFE mutations positive patients, depending on the genotype, and represented the predominant pattern of iron accumulation in approximately one third of them (range, 29.8%–35.7%). On the other hand, predominantly hepatocellular iron accumulation occurred in patients carrying HFE mutations in 52.7% of cases but only in 19.1% of cases in those positive for the C282Y/wt, H63D/H63D, and C282Y/H63D genotypes (Figure 2). These 3 genotypes accounted for 22.8% of predominantly nonparenchymal iron accumulation.

Table 2.

Frequency Distribution of *HFE* Genotypes in 587 Italian Patients With NAFLD and 184 Healthy Subjects With Normal Liver Enzymes and Metabolic Parameters, *P* = .6

	HFE genotype				
	wt/wt	H63D/wt	C282Y/wt	H63D/H63D	C282Y/H63D
Patients, n = 587 (*%*)	367 (62.5)	152 (25.9)	34 (5.8)	19 (3.2)	15 (2.5)
Controls, n = 184 (*%*)	114 (62.0)	53 (28.8)	8 (4.4)	7 (3.8)	2 (1.1)
Adjusted OR[a]	Reference	0.65	1.37	0.70	2.42
95% CI	Reference	0.40−1.03	0.63−3.16	0.30−1.75	0.82−11.02

a

Adjusted for age, sex, and BMI.

Table 3.

Effect of *HFE* Genotypes on Serum Iron Parameters in 587 Italian Patients With NAFLD and of Hepatic Siderosis in 480 Patients

	HFE genotype					*P* value
	wt/wt (n = 367)	H63D/wt (n = 152)	C282Y/wt (n = 34)	H63D/H63D (n = 19)	C282Y/H63D (n = 15)	
Serum iron parameters in 587 NAFLD patients						
Ferritin (*ng/mL*)	223 {123–530}	266 {128–580}	532 {168–784}[a]	545 {169–715}[a]	458 {298–515}[a]	.005
Transferrin saturation (*%*)	32.8 ± 11.7	35.9 ± 13.7[a]	40.7 ± 13.7[a]	39.0 ± 12[a]	38.7 ± 8.8[a]	.0007
Hepatic siderosis, 480 patients	wt/wt (n = 292)	H63D/wt (n = 124)	C282Y/wt (n = 33)	H63D/H63D (n = 17)	C282Y/H63D (n = 14)	
Hepatocellular siderosis (*%*)	95 (32.5)	53 (42.7)	15 (45.5)	13 (76.5)[a]	9 (64.3)[a]	.0004
Kupffer cell siderosis (*%*)	79 (27.1)	33 (26.6)	15 (45.5)[a]	11 (64.7)[a]	8 (57.1)[a]	.0005
Overall siderosis grade >2	108 (29.4)	49 (32.2)	18 (52.9)[a]	14 (73.9)[a]	12 (75.0)[a]	<.0001
Siderosis pattern						<.0001
No siderosis	168 (57.5)	64 (51.6)	12 (36.4)[a]	2 (11.8)[a]	1 (7.1)[a]	<.0001
Hepatocellular	52 (17.8)	37 (29.8)[a]	9 (27.3)	7 (41.2)[a]	5 (35.7)	.01
Nonparenchymal	72 (24.7)	23 (18.6)	12 (36.4)	8 (47.0)	8 (57.2)[a]	.002

NOTE. Results are expressed as mean ± standard deviation except that for ferritin, where median {interquartile range} is reported.

a

P < .05 vs wt/wt.

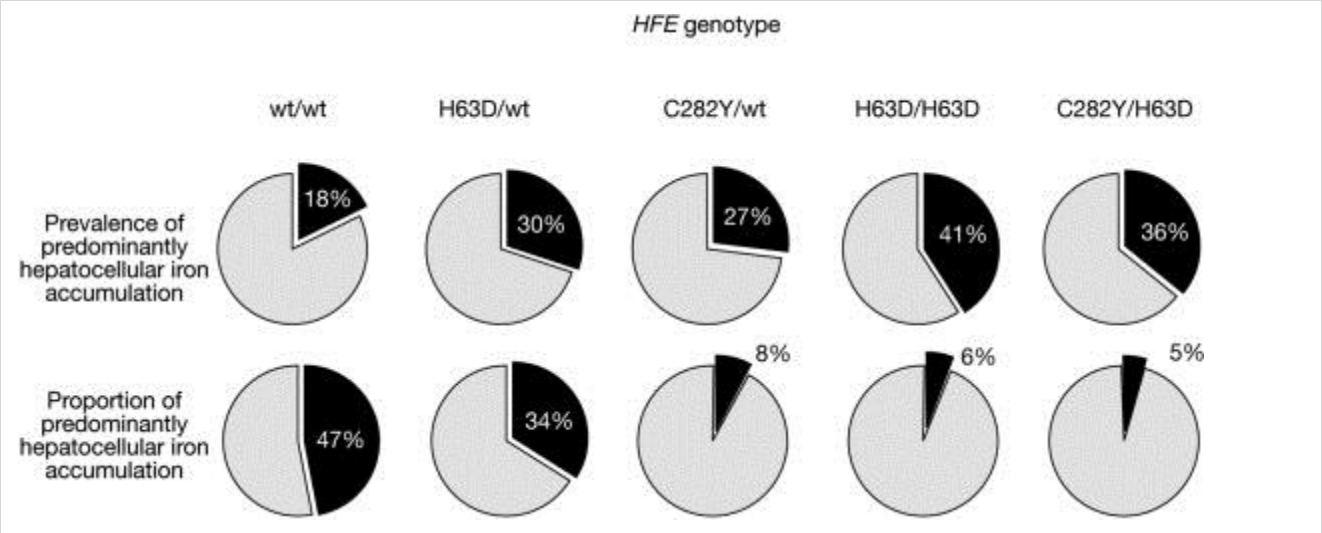

Figure 2.

Penetrance of predominantly parenchymal iron overload (ie, the percentage of subjects with a given *HFE* genotype presenting with predominantly parenchymal iron accumulation) and relative contribution to the whole burden of predominantly parenchymal iron accumulation (ie, the percentage of subjects with predominantly parenchymal iron accumulation attributable to a given *HFE* genotype) in 480 patients with NAFLD subdivided according to *HFE* genotypes. Wt, wild-type.

The sensitivity and specificity of predominantly parenchymal iron accumulation according to the presence of HFE mutations were 52.7% and 64.8%, respectively. The association between the presence and pattern of hepatic iron accumulation and moderate/severe fibrosis (stage >1) adjusted for other risk factors (age, BMI, presence of diabetes, and ALT levels) is shown in Table 4. The presence of predominantly hepatocellular iron accumulation was associated with a 1.68-fold higher risk of fibrosis >1 (95% confidence interval [CI]: 1.2–2.3) compared with the absence of detectable siderosis, an amount of risk very similar to that conferred by the presence of diabetes and prediabetes. In contrast, the presence of predominantly nonparenchymal siderosis was not associated with increased fibrosis >1, with an OR nearly associated with a protective effect (OR, 0.72; 95% CI: 0.5–1.01).

Table 4.

Independent Predictors of Fibrosis >1 at Logistic Regression Analysis in 480 Patients With NAFLD With Histologic Characterization of Hepatic Iron Deposition

	Odds ratio	95% CI	*P* value
Age, *y*	1.023	1.003–1.044	.023
BMI (*kg/m*^{2})	1.093	1.029–1.162	.004

	Odds ratio	95% CI	*P* value
Diabetes/IFG/IGT	1.579	1.243–2.005	.0002
ALT, *IU/mL*	1.011	1.007–1.016	<.0001
Siderosis pattern			
None	Reference	—	—
Hepatocellular	1.684	1.210–2.343	.002
Nonparenchymal	0.719	0.503–1.011	.063

IFG, impaired fasting glucose; IGT, impaired glucose tolerance.

At logistic regression analysis adjusted for age, BMI, presence of hyperglycemia, and ALT levels, fibrosis >1 was also associated with higher transferrin saturation (OR, 1.017 for each unit increase in percentage transferrin saturation, 95% CI: 1.001–1.034; P = .033), known to be associated with parenchymal iron deposition, but not with ferritin levels (OR, 1.003; 95% CI: 0.999–1.001; P = .33), reflecting both parenchymal and nonparenchymal iron accumulation, or the global severity of histologic siderosis independently of iron compartmentalization (OR, 1.089; 95% CI: 0.911–1.296; P = .346).

At univariate analysis, both hepatocellular and nonparenchymal siderosis were significantly associated (P < .05) with male sex, higher transferrin saturation percentage, higher serum ferritin, and lower ALT levels (not with BMI levels). Hepatocellular siderosis was also significantly associated with lower platelets levels, higher total bilirubin, and lower percentage of hepatocytes with fat droplets. Nonparenchymal siderosis was also significantly associated with older age and higher platelet levels (Table 5). Serum ferritin was significantly higher in patients with nonparenchymal than in those with predominantly parenchymal iron deposition (P < .05).

Table 5.
Clinical Variables Associated With the Pattern of Hepatic Iron Accumulation in 480 Italian Patients With NAFLD

	Hepatic iron deposition			*P* value
	Hepatocellular (n = 110)	Nonparenchymal (n = 123)	None (n = 247)	
Age, *y*	46.3 ± 11	48.9 ± 11[a]	44.6 ± 11	.003
Sex, F	15 (13.6)[a]	17 (13.8)	56 (22.7)	.040
Transferrin saturation, *%*	37.5 ± 14[a]	38.9 ± 11[a]	30.9 ± 12	<.0001
Ferritin, *ng/mL*	354 {177–578}[a]	606 {399–832}	166 {89–289}	<.0001
ALT, *IU/mL*	60.9 ± 35[a]	61.3 ± 45[a]	74.0 ± 52	.014

	Hepatic iron deposition			
				P value
	Hepatocellular (n = 110)	Nonparenchymal (n = 123)	None (n = 247)	
Platelets, *10^3/dL*	119.5 ± 113[a]	187.8 ± 97[a]	170.8 ± 112	<.0001
Bilirubin, *mg/dL*	1.03 ± 0.5[a]	0.96 ± 0.5	0.84 ± 0.5	.008
Steatosis, *%* [b]	24.8 ± 22[a]	36.1 ± 24	34.1 ± 27	.026

NOTE. Results are expressed as mean ± standard deviation except that for ferritin, where median {interquartile range} is reported.

a

P < .05 vs patients without iron deposition.

b

Percent of fat-laden hepatocytes evaluated on histologic sections.

Because HFE mutations predispose to hepatic iron accumulation and predominantly parenchymal iron accumulation was independently associated with the presence of moderate/severe fibrosis, we next determined whether HFE mutations predisposed to liver damage. HFE genotypes were not significantly associated with demographic, anthropometric, metabolic features, and liver enzymes in this series. The prevalence of liver fibrosis stage >1 and >2 according to HFE genotypes is shown in Figure 3A. There was no significant association between HFE genotype, either considering each separate genotype or only the presence of C282Y and H63D mutations, and the severity of liver fibrosis in the whole series of subjects analyzed, as well as in the 480 subjects in whom the evaluation of hepatic siderosis was available (not shown in detail). Also restricting the analysis to subjects with BMI <30 or <27.5 kg/m2, HFE genotype was not associated with fibrosis >1, whereas parenchymal siderosis was significantly associated with fibrosis >1 in these subgroups. The prevalence of fibrosis >1 was higher in patients with hepatocellular iron accumulation independent of the HFE genotype (Figure 3B).

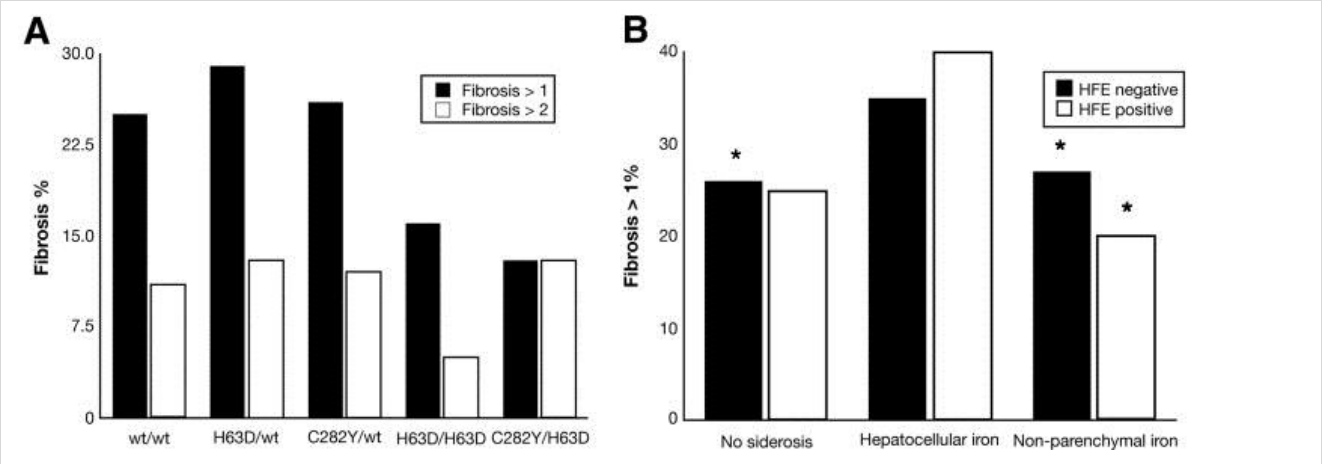

Figure 3.

(*A*) Prevalence of fibrosis stage >1 and >2 in 587 Italian patients with NAFLD subdivided according to the *HFE* genotype. Wt, wild-type. (*B*) Prevalence of fibrosis stage >1 in 480 Italian patients with NAFLD subdivided according to the presence/pattern of hepatic iron deposition and the presence of the H63D or C282Y HFE mutations. *$P < .05$ vs patients positive for *HFE* mutations and predominantly hepatocellular iron deposition.

Discussion

Altered iron parameters are frequently detected in patients with NAFLD,16 and parenchymal iron accumulation is a well-recognized and treatable determinant of liver damage in patients with hereditary hemochromatosis.22 Because HFE mutations are the main cause of hemochromatosis and parenchymal iron accumulation in white ethnicity individuals,21 several studies have investigated the role of the common C282Y and H63D HFE genetic variants in the progression of liver damage in NAFLD, which has a strong genetic component. 11

Conflicting data have been reported on the role of HFE mutations on liver damage in NAFLD. Some studies suggested an association between the presence of the C282Y mutation and other HFE genotypes predisposing to iron overload with more severe liver damage, 26 and 30 whereas others did not detect any effect on the severity of liver fibrosis.

In the present study, we found that hepatocellular, but not nonparenchymal iron deposition, was a risk factor for moderate/severe fibrosis independently of confounding variables in a large series of Italian patients with NAFLD. These findings are in line with previous results26 and 30 and fit well with the known association between the severity and duration of parenchymal iron overload and the progression of liver fibrosis in patients with hereditary hemochromatosis, as well as with the lack of any association between nonparenchymal iron accumulation because of mutations inactivating the iron exporter ferroportin and liver fibrosis.22 and 31

However, different from our results, in a large multiethnic series of NAFLD patients from the United States, Nelson et al have recently presented preliminary results indicating that nonparenchymal, but not predominantly hepatocellular, iron accumulation was independently associated with more advanced liver damage.32 Unfortunately, the genetic characterization of these subjects was not reported. The apparent discrepancy in the association between the pattern of iron overload and fibrosis may be at least partially explained by ethnic differences because of different genetic factors responsible for iron overload and fibrosis and by the significantly lower body mass observed in US patients with predominantly parenchymal iron accumulation. Interestingly, we also observed a nonsignificant trend for lower BMI and steatosis percentage and lower ALT levels in patients with predominantly parenchymal iron accumulation.

Even though HFE mutations increased the risk of parenchymal iron accumulation, they were not associated with liver damage. The most likely explanation of these findings is related to the weak association between HFE mutations and hepatocellular iron deposition. Indeed, only 30%–41% of patients carrying HFE mutations had phenotypically expressed predominantly parenchymal iron accumulation, as compared with 18% of subjects negative for HFE mutations, and 47% of patients with predominantly hepatocellular iron accumulation did not carry HFE mutations. These data are not surprising because it has recently been recognized that the penetrance of HFE mutations, even that of homozygosity for the C282Y, is very low, with as few as 1%–20% of affected individuals developing overt liver disease. 33 Thus, based on the data obtained in the general population, we did not expect a high penetrance of certain HFE genotypes, such as heterozygosity for the H63D and C282Y mutations, on parenchymal iron accumulation. 21

NAFLD by itself was reported to predispose to liver iron accumulation,34 but, as confirmed in this series, the most typical patterns are either nonparenchymal or mixed hepatic iron deposition, which may be related to liver cell necrosis, inflammation, altered cytokines release (possibly the cause of higher platelet count in patients with nonparenchymal iron deposition), and down-regulation of protein involved in cellular iron export.35 Unexpectedly, nonparenchymal iron deposition was more common than hepatocellular iron deposition in subjects with HFE genotypes at risk, further contributing to the lack of association between HFE mutations and more advanced liver damage. Thus, as in hereditary hemochromatosis, additional, possibly ethnic-specific, largely unknown genetic factors are likely to modify the penetrance of HFE mutations on parenchymal iron accumulation in NAFLD and to contribute to parenchymal iron deposition in patients negative for HFE mutations.

As an example, it was previously reported in a subset of this series14 that α1-antitrypsin mutations induced a shift in the pattern of iron accumulation toward nonparenchymal cells and were protective against parenchymal iron accumulation and liver fibrosis in patients carrying HFE mutations. Furthermore, it is possible that hemoglobin defects play a role because, in Italy, the β-thalassemia trait was associated with hepatic iron accumulation (because of ineffective erythropoiesis, suppression of hepcidin release, and increased iron absorption) and liver fibrosis in patients with chronic HCV infection. 36 The higher total bilirubin levels observed in patients with hepatocellular siderosis may be due to ineffective erythropoiesis or to more severe liver disease, in line with the higher severity of fibrosis and lower platelet count. However, the HFE modifier genes hypothesis needs to be proven in further studies, and a majority of the involved genes are probably still unknown.

Despite the aforementioned limitations, this study also has strengths. It considers a case series much larger than any previous one, recruited in different centers from Northern Italy, of which only 1 represented a referral center for iron overload disorders, with a genetically homogenous background. A DNA sample and consent for determination of HFE genotype were available for the majority of subjects submitted to liver biopsy at the different centers, the characterization of serum iron parameters was available for all patients, and there was a blinded re-evaluation of hepatic siderosis for a large majority of them.

In conclusion, only predominantly hepatocellular iron deposition is associated with more severe liver damage in Italian patients with NAFLD. However, HFE genotype determination is not clinically useful in these patients, unless evidence of severe parenchymal iron accumulation has been obtained, because by itself it is not sufficient to identify patients with hepatocellular iron. Additional studies are required to evaluate the role of HFE modifier genes on iron metabolism and liver damage in patients with NAFLD.

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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.

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