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ORIGINAL ARTICLE

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Hemochromatosis and transferrin receptor gene polymorphisms in chronic hepatitis C: impact on iron status, liver injury and HCV genotype

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Abstract Mild iron overload in chronic hepatitis C is associated with liver fibrosis, hepatitis C virus (HCV) genotype 1b infection, and an impaired response to interferon therapy. In this study we evaluated whether polymorphisms in the hemochromatosis gene *HFE* and the transferrin receptor gene *TFR1* are associated with these typical findings. The study considered 246 HCV-infected patients and 200 blood donors as controls, in which C282Y, H63D, and S65C mutations (*HFE*) and the S142G polymorphism (*TFR1*) were detected. HCV genotype, serum ferritin levels, stainable intrahepatic iron, and grade of fibrosis according to the METAVIR score (F0–F4) were determined. In HCV-infected patients, heterozygosity for the C282Y mutation in *HFE* was significantly associated with elevated serum ferritin levels, stainable liver iron, and advanced fibrosis or cirrhosis (F2–F4). By multivariate logistic regression analysis the odds ratio for the development of advanced fibrosis or cirrhosis (F2–F4) was 2.5 for HCV-infected patients carrying a heterozygous C282Y mutation and 4.8 for HCV-infected patients with C282Y/H63D and C282Y/S65C compound heterozygosity. Heterozygosity for the C282Y mutation in *HFE* contributes to iron accumulation and fibrosis progression in chronic hepatitis C.

Keywords Hepatitis C · Fibrosis · Siderosis · Hemochromatosis · Genetic polymorphisms

Abbreviation *HCV:* Hepatitis C virus

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Introduction

Chronic hepatitis C virus (HCV) infection is frequently associated with elevated serum ferritin levels, and iron accumulation in liver biopsies [1, 2, 3, 4]. In the past decade there has been growing interest in this topic because the amount of iron accumulation is correlated with inflammatory activity and grade of fibrosis in HCV-infected patients [3, 5, 6]. Moreover, iron overload in chronic HCV infection has been reported to result in an impaired response to interferon therapy [7, 8, 9], and thus therapeutic phlebotomies may improve the treatment response [10].

The pathogenesis of hepatic iron accumulation in chronic HCV infection is still unclear. Heterozygosity for hereditary hemochromatosis, an autosomal recessive iron storage disease leading to progressive organ damage including the liver, has been discussed as an important cofactor for HCV-associated hepatic iron accumulation

[1, 11]. The identification of the hemochromatosis gene *HFE* by Feder et al. [12] has strengthened the ability to diagnose the disease accurately. More than 90% of patients with hereditary hemochromatosis carry a homozygous cysteine-to-tyrosine substitution at amino acid position 282 in HFE (C282Y mutation) [12, 13]. Additionally, a substitution at amino acid position 63 (H63D mutation) may result in hemochromatosis when coinherited with the C282Y mutation. This compound heterozygote state accounts for a low percentage of hemochromatosis cases [13].

HFE mutations, however, are also involved in the pathogenesis of chronic liver diseases other than hereditary hemochromatosis. Several studies demonstrated a significantly higher prevalence of homozygous and heterozygous C282Y mutations in patients with nonalcoholic steatohepatitis [14] and porphyria cutanea tarda [15, 16]. In contrast, the role of *HFE* mutations in the natural course of chronic hepatitis C remains controversial. While Smith et al. [17], Bonkovsky et al. [18], Tung et al. [19], and Erhardt et al. [20] found an association of the C282Y mutation with hepatic iron accumulation and advanced fibrosis in HCV-infected patients, other investigators have not confirmed this finding [6, 21, 22, 23, 24].

In addition, a functional association between the HFE protein and the transferrin receptor (*TFR1*) has recently been demonstrated. Wild-type HFE binds in vitro to homodimeric transferrin receptors and lowers the affinity for iron-saturated transferrin, resulting in a decreased cellular uptake of transferrin bound iron [25, 26]. Although polymorphisms in *TFR1* have not yet been demonstrated to induce iron overload, a frequent polymorphism in *TFR1*, resulting in a glycine for serine substitution on position 142 (S142G polymorphism) seems to influence tumor progression. Van Landeghem et al. [27, 28, 29] found that homozygosity for the *TFR1* S142 allele in combination with the C282Y mutation in *HFE* results in an increased risk for developing various neoplasms, such as multiple myeloma as well as breast, colorectal, and hepatocellular carcinomas.

Taken together, previous data suggest that both *TFR1* and *HFE* gene products influence the course of diseases that are susceptible to intracellular iron content. Therefore the aims of our study were to evaluate the influence of genetic polymorphisms in *HFE* and *TFR1* on iron accumulation and on prognostic factors such as liver fibrosis in a large group of HCV-infected patients.

Patients and methods

Patients

All patients with chronic HCV infection seen in our outpatient clinic in the period from February 1998 to January 2000 prior to interferon therapy were evaluated as possible candidates for this study. Patients with excessive alcohol consumption (daily alcohol consumption or more than 7 drinks/week) or any other drug abuse including methadone substitution were excluded. Additional exclusion criteria were the diagnosis of hereditary hemochromatosis, blood transfusions during the past 5 years, anemia, other liver diseases such as infection with hepatitis B virus, and autoimmune liver disease. As demonstrated in Table 1, 246 individuals met all criteria and were enrolled (151 men, and 95 women; mean age 42 years, range 17–72). The source of infection was transfusion in 70 (28.5%), intravenous drug use in 82 (33.3%), and sporadic or unknown in 94 (38.2%) HCV-infected patients. All patients had elevated serum transaminase levels for at least 6 months and were positive for antibodies to HCV (third-generation anti-HCV enzyme-linked immunosorbent assay; Abbott, Wiesbaden, Germany) and HCV RNA as detected by polymerase chain reaction (Amplicor system; Roche Diagnostics, Grenzach-Whylen, Germany). HCV genotype was determined in all patients by a commercially available line-probe assay (Inno-Lipa; Innogenetics, Zwijndrecht, Belgium). Routine biopsy was performed in 222 patients (90%) for exact staging of the disease. Grade of fibrosis was determined according to the METAVIR score system (F0=no fibrosis, F1=portal fibrosis without septa, F2=portal fibrosis with rare septa, F3=numerous septa without cirrhosis, and F4=cirrhosis) [30]. In addition, a semiquantitative histological grading was used for the histological assessment of liver iron accumulation upon Perls' staining. Serum ferritin levels (measured by ECLIA technology on an Elecsys analyzer, Roche Diagnostics, Mannheim, Germany) were available from 231 of the 246 patients. The upper normal limit for serum ferritin was defined as 300 µg/l in men and 200 µg/l in women. Transferrin saturation was calculated from serum iron (determined photometrically on an LX-Analyzer, Beckman-Coulter, Krefeld, Germany) and serum transferrin (determined by nephelometry on a BNAII analyzer, Dade-Behring, Schwalbach, Germany) as iron (µg/dl) × 100/transferrin (mg/dl) × 1.4 in 213 of 246 patients. The upper normal limit for transferrin saturation was defined as 45%. In patients receiving interferon therapy liver biopsy was performed and serum iron index determined before initiation of therapy.

The control group consisted of 200 randomly selected healthy blood German donors from the area of Heidelberg, Germany. Our blood donors serving as healthy controls had similar exclusion criteria as the HCV-infected patients. The ratio of aspartate aminotransferase to alanine aminotransferase was determined prior to each blood donation, as well as the hepatitis B surface antigen, anti-hepatitis B core antigen, anti-HCV, and anti-HIV1/2 status. Samples were received under code, and the identity remained unknown. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the local ethics committee. Informed consent was obtained from all patients prior to liver biopsy and genotype analysis.

Table 1 Clinical data from HCV-infected patients and controls

	Patients (*n*=246)	Controls (*n*=200)
Sex: M/F	151/95	101/99
Age (years)	42±12	39±10
Age range (years)	17–72	18–58
HCV genotype		
Type 1	174 (70.7%)	–
Type 2	22 (8.9%)	–
Type 3	46 (18.7%)	–
Type 4	4 (1.6%)	–
Stainable iron in liver biopsy	13 (5.3%)	–
Fibrosis in liver biopsy[a]		
Grade 0	136 (55.3%)	–
Grade 1	33 (13.4%)	–
Grade 2	15 (6.1%)	–
Grade 3	14 (5.7%)	–
Grade 4	24 (9.8%)	–
Liver biopsy not done	24 (9.8%)	–

[a] METAVIR score

Table 2 Primer and hybridization probes for detection of the S142G polymorphism in *TFR1* and for dual-color detection of the C282Y, H63D, and S65C mutations in *HFE* (*underlined* mismatches between mutation probes and corresponding alleles)

Oligonucleotide	Sequence	Orientation	Position[a]
TFR1 (*S142G*)			
TFR-501	5′-CTG CAG CAC GTC GCT TAT ATT G-3′	Sense	350–371
TFR-301	5′-GTT CTA CCT TTT CCC CTA CCA GTA TAG-3′	Antisense	Intron
TFR-LCM	5′-CAC AGA CTT CAC CGG CAC CAT CAA-fluorescein	Sense	411–434
TFR-LCA	5′-LC Red 640-GAG TGC CAG CTG CTG TGC AAG TAT CTA GAC AAG TAA TTC A-p	Sense	Intron
HFE (*C282Y*)			
C282Y-501	5′-TGG CAA GGG TAA ACA GAT CC-3′	Sense	Intron
C282Y-301	5′-TAC CTC CTC AGG CAC TCC CC-3′	Antisense	Intron
C282Y-LCM	5′-AGA TAT ACG TAC CAG GTG GAG-fluorescein	Sense	835–855
C282Y-LCA	5′-LC Red 640-CCC AGG CCT GGA TCA GCC CCT CAT TGT GAT CTG GG-p	Sense	858–892
HFE (*H63D/S65C*)			
S65C-501	5′-GCT CTG TCT CCA GGT TCA CAC TC-3′	Sense	Intron
S65C-301	5′-CCC TCT CCA CAT ACC CTT GC-3′	Antisense	Intron
S65C-LCM	5′-CGG CGA CTC TCA TCA TCA TAG AAC ACG AAC A-fluorescein	Antisense	200–170
S65C-LCA	5′-LC Red 705-CTG GTC ATC CAC GTA GCC CAA AGC TTC AA-p	Antisense	168–140

[a] According to GenBank accession no. XM_052730 (*TFR1*) and U60319 (*HFE*)

Mutation analysis

For mutation analysis genomic DNA was isolated using the QIAamp blood kit (Qiagen, Hilden, Germany) from whole blood. Analysis of the *TFR1* S142G polymorphism was carried out on a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) as described in detail for other polymorphisms [31]. Briefly, PCR was performed by cycling with the primers TFR-501 and TFR-301 and two fluorescently labeled hybridization probes (TIB Molbiol, Berlin, Germany) TFR-LCM (mutation probe) and TFR-LCA (anchor probe; Table 2). For simultaneous detection of the C282Y, H63D, and S65C mutations in *HFE* we developed a multiplex dual-color PCR assay. A detailed description of the method is available at http://download.gehrke.net/HFE-LightCycler.pdf. Briefly, the C282Y loci were amplified using the primers C282Y-501 and C282Y-301. The hybridization probes used to detect the C282Y mutation were anchor probe C282Y-LCA and mutation probe C282Y-LCM (Table 2). The H63D and S65C loci were amplified in the same capillary using the primers S65C-501 and S65C-301. Hybridization probes for detection of the H63D and S65C mutations were anchor probe S65C-LCA and mutation probe S65C-LCM (Table 2). During the PCR and melting curve generation fluorescence was monitored in different channels. Channel 2 (LightCycler Red 640) was used for genotyping the C282Y mutation and channel 3 (LightCycler Red 705) was used for genotyping the H63D and S65C mutations.

Statistical analysis

Fisher's exact test, the χ^2 test, the nonparametric Mann-Whitney test, and logistic regression were used to analyze the data. In addition, Bonferroni's correction was used for multiple testing. A P value less than 0.05 was considered statistically significant. All associations remained statistically significant after Bonferroni's correction for multiple testing, unless otherwise mentioned in the manuscript. Statistical analyses were performed using StatView Version 5.0 (SAS Institute Cary, N.C., USA).

Results

The HCV genotype 1 was found in the majority of the 246 patients (71%) , followed by genotype 3 which was found in 48 (19%) patients (Table 1). Of the 222 patients in whom liver biopsies were performed 169 (76%) showed no or mild fibrosis (METAVIR score F0 or F1), and only 53 of the 222 (24%) had progressive fibrosis or cirrhosis (METAVIR score F2–F4).

HFE and *TFR1* genotypes were determined in all patients and controls (Table 3). Allele frequencies of *HFE* mutations in patients and controls were 0.061 and 0.04 for the C282Y mutation, 0.134 and 0.123 for the H63D mutation, and 0.012 and 0.015 for the S65C mutation. The allele frequency of the S142G polymorphism in *TFR1* was 0.439 in HCV-infected patients compared with 0.460 in healthy controls. Neither genotypes (Table 3) nor allele frequencies differed significantly between patients and controls. In both patients and controls there was no subject homozygous for either the C282Y or the S65C mutation.

As shown in Fig. 1, serum ferritin levels were significantly higher in HCV-infected patients with stainable liver iron ($P<0.0001$), extensive liver fibrosis grade F2–F4 ($P<0.001$), and HCV genotype 1b infection ($P<0.01$) than in HCV-infected patients without those clinical features. According to *HFE* genotype, only heterozygosity for the C282Y mutation ($P<0.01$) and homozygosity for the H63D mutation ($P<0.01$) were found to be associated with increased serum ferritin levels. Apart from homozygosity for the H63D mutation, all associations remained statistically significant after Bonferroni's correction for multiple comparisons. Neither a heterozygous H63D mutation nor a S65C mutation in *HFE* had a significant impact on serum ferritin levels in HCV-infected patients, even when inherited simultaneously with the C282Y mutation in a compound heterozygote state. The S142G polymorphism in *TFR1* was also not associated with changes in serum ferritin levels.

Table 3 Distribution of *HFE* and *TFR1* genotypes in 246 HCV-infected patients and 200 controls

			Patients (*n*=246)		Controls (*n*=200)	
			n	%	*n*	%
HFE genotype						
C282Y +/+	H63D –/–	S65C –/–	0	–	0	–
C282Y +/–	H63D +/–	S65C –/–	6	2.4	4	2
C282Y +/–	H63D –/–	S65C +/–	2	0.8	0	
C282Y +/–	H63D –/–	S65C –/–	22	8.9	12	6
C282Y –/–	H63D +/+	S65C –/–	5	2	2	1
C282Y –/–	H63D +/–	S65C +/–	2	0.8	1	0.5
C282Y –/–	H63D +/–	S65C –/–	48	19.5	40	20
C282Y –/–	H63D –/–	S65C +/+	0	–	0	–
C282Y –/–	H63D –/–	S65C +/–	2	0.8	5	2.5
C282Y –/–	H63D –/–	S65C –/–	159	64.6	136	68
TFR1 genotype						
S142G +/+	–	–	41	16.7	44	22
S142G +/–	–	–	134	54.5	96	48
S142G –/–	–	–	71	28.9	60	30

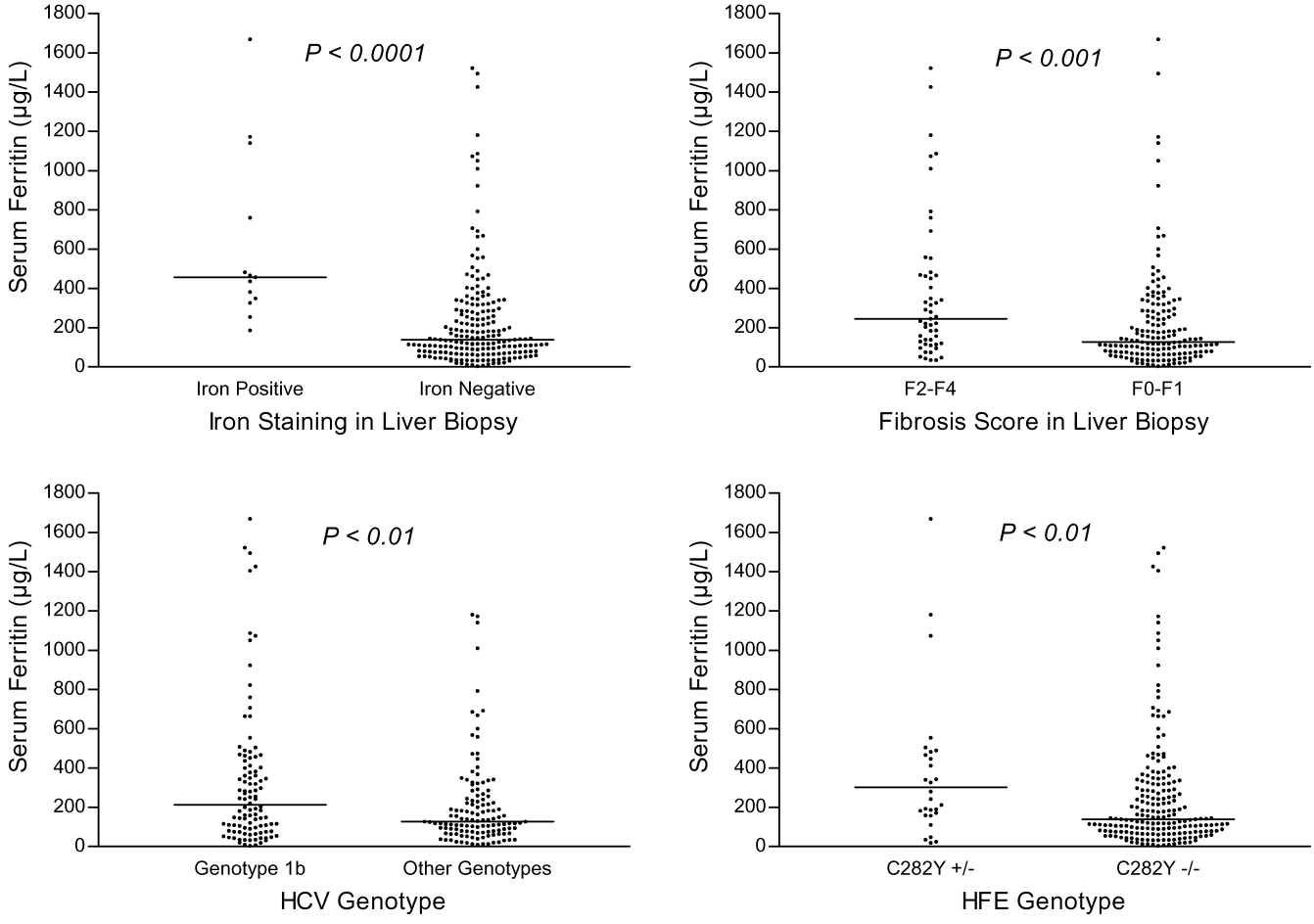

Fig. 1 Association of serum ferritin levels with histological parameters (stainable iron and fibrosis score), HCV genotype 1b, and heterozygosity for the C282Y mutation in *HFE* among HCV-infected patients. *Black dots* Values of individual patients; *solid lines* median values: 456 µg/l (iron positive in liver biopsy) vs. 139 µg/l (iron negative in liver biopsy), 245 µg/l (fibrosis score F2–F4) vs. 127 µg/l (fibrosis score F0-F1), 212 µg/l (HCV genotype 1b) vs. 127 µg/l (other HCV genotypes), and 303 µg/l (heterozygous C282Y mutation) vs. 138 µg/l (homozygous wild-type at the C282Y locus). All graphically presented data remained statistically significant after Bonferroni's correction for multiple comparisons ($P<0.05$). Homozygosity for the H63D mutation in *HFE* was also associated with increased serum ferritin levels (median 923 µg/l vs. 147 µg/l; $P<0.01$), but without statistical significance after Bonferroni's correction for multiple comparisons

Table 4 *HFE* and *TFR1* polymorphisms in patients with chronic HCV infection. allele frequency and odds ratio for advanced liver fibrosis (F0, F1 vs. F2–F4)

Polymorphism	Allele frequency					Multivariate regression analysis[a]	
	F0, F1 (*n*=338)		F2–F4 (*n*=106)		*P*	Odds ratio (95% CI)	*P*
	n	%	*n*	%			
C282Y (*HFE*)	15	4.4	12	11.3	<0.01[b]	2.6 (1.1–6.4)	<0.05
H63D (*HFE*)	45	13.3	13	12.3	n.s.	0.8 (0.4–1.8)	n.s.
S65C (*HFE*)	4	1.2	2	1.9	n.s.	2.1 (0.3–14.4)	n.s.
S142G (*TFR1*)	151	44.7	44	41.5	n.s.	1.2 (0.6–2.6)	n.s.

[a] Further parameters included for analysis were age (>50 vs. ≤ 50 years), sex, previous alcohol consumption, macrovesicular steatosis in liver biopsy specimen, HCV genotype (1, 4 vs. 2, 3), and *HFE* and *TFR1* polymorphisms
[b] After Bonferroni's correction for multiple comparisons

Table 5 Multivariate logistic regression analysis[a] for advanced liver fibrosis (F0–F1 vs. F2-F4) in patients with chronic HCV infection and *HFE* mutations compared with HCV-infected patients carrying a homozygous wild-type *HFE* allele

	n	Odds ratio (95% CI)	*P*
Any *HFE* mutation (all genotypes except WT/WT)	222	1.3 (0.7–2.5)	n.s.
C282Y heterozygosity (C282Y/WT, C282Y/H63D, C282Y/S65C)	173	2.5 (1.0–6.3)	<0.05
C282Y compound heterozygosity (C282Y/H63D, C282Y/S65C)	154	4.8 (1.0–22.3)	<0.05
H63D heterozygosity/homozygosity (H63D/WT, H63D/H63D)	191	0.8 (0.3–1.9)	n.s.

[a] Further parameters included for analysis were age (>50 vs. ≤ 50 years), sex, previous alcohol consumption, macrovesicular steatosis in liver biopsy specimen, HCV genotype (1, 4 vs. 2, 3), and *TFR1* genotype (presence of the S142G polymorphism)

Stainable iron in liver biopsy specimens was significantly associated with heterozygosity for the C282Y mutation. Of 13 patients with stainable liver iron 5 (38.5%) were heterozygous for the C282Y mutation. In contrast, 22 of 209 (10.5%, $P<0.01$) patients without stainable liver iron carried the C282Y mutation.

An association of grade of fibrosis with heterozygosity for the C282Y mutation was also found. Of the 53 HCV-infected patients with progressive fibrosis (F2–F4) 12 (22.6%) were heterozygous for the C282Y mutation. In contrast, only 15 of 169 patients (8.9%, $P<0.01$) with fibrosis grade F0 or F1 carried the C282Y mutation. Using a multivariate logistic regression analysis including sex, age, previous alcohol consumption, macrovesicular steatosis in liver biopsy, HCV genotype, and the presence of *HFE* and *TFR1* polymorphisms as variables, the C282Y mutation was identified as an independent factor for development of advanced liver fibrosis (F2–F4) (Table 4). The odds ratio for fibrosis grade F2–F4 was 2.5 ($P<0.05$) for HCV-infected patients carrying a heterozygous C282Y mutation (including compound heterozygotes) compared with HCV-infected patients carrying a homozygous wild-type allele. For C282Y compound heterozygous HCV-infected patients (C282Y/H63D, C282Y/S65C), the odds ratio for development of liver fibrosis grade F2–F4 increased to 4.8 ($P<0.05$; Table 5).

Of 114 patients with a HCV genotype 1b infection 19 (16.7%) carried a heterozygous C282Y mutation. In comparison, of the 132 patients infected with HCV genotypes other than 1b only 11 (8.3%; $P<0.05$) were C282Y heterozygotes. The combination of heterozygosity for the C282Y mutation and homozygosity for the S142 allele in *TFR1* was found in 10/114 (8.8%) patients with HCV genotype 1b. In contrast, only 2/132 (1.5%; $P<0.05$) patients infected with HCV genotypes other than 1b carried this *HFE/TFR1* genotype combination. Using multivariate logistic regression analyses including sex, age, and fibrosis as variables, these associations did not remain statistically significant.

Discussion

During the past decade heterozygosity for the autosomal recessive iron storage disease hemochromatosis has been discussed as an important cofactor for hepatic iron accumulation and disease progression in HCV-infected patients [1, 11]. The discovery of the hemochromatosis gene, *HFE*, by Feder et al. [12] improved the definition of heterozygosity for the disease. However, recent studies in which *HFE* mutation analyses were performed in HCV-infected patients produced discrepant results (Table 6). Of the studies published between 1998 and 2002 [6, 17, 18, 21, 22, 23, 24] only two that included a limited number of HCV-infected patients [17, 18] demonstrated a statistical significant association between heterozygosity for hemochromatosis (heterozygous C282Y mutation in *HFE*) and progression of liver fibrosis. As this possible association is a most controversial issue, further studies with adequate statistical methods including logistic regression analysis are required [32]. Remarkably, two recent studies that fulfilled these criteria and included a large number of HCV-infected patients demonstrated an association between *HFE* mutations and hepatic iron accumulation and fibrosis progression [19, 20].

In the present study we analyzed the role of genetic polymorphisms in *HFE* and *TFR1* in a large number of

Table 6 Summary of studies evaluating the role of *HFE* mutations in chronic HCV infection (*n* number of patients with liver biopsy performed, *NA* not available, *HIC* hepatic iron concentration)

	n	Method of hepatic iron determination	Association between *HFE* mutations and hepatic iron accumulation	Association between *HFE* mutations and liver injury	Specific comments
Smith et al. [17]	137	Perl's staining	Higher prevalence of C282Y heterozygosity in iron stain positive patients ($P<0.05$)	Higher prevalence of liver cirrhosis in C282Y heterozygotes ($P<0.05$)	Higher serum ferritin levels in C282Y heterozygotes ($P<0.001$)
Hézode et al. [6]	209	Perl's staining	No association	NA	Patients with chronic active hepatitis
Kazemi-Shirazi et al. [21]	149	Perl's staining HIC ($n=114$)	No association	No association	
Martinelli et al. [22]	102	Perl's staining	No association	Higher grade of liver fibrosis in patients with any *HFE* mutation ($P<0.05$)	Higher transferrin saturation in patients with any *HFE* mutation ($P<0.001$)
Negro et al. [23]	120	Perl's staining	No association	No association	HCV genotype 3 less frequent in iron stain positive patients ($P<0.05$)
Thorburn et al. [24]	164	Perl's staining HIC ($n=120$)	No association	No association	
Bonkovsky et al. [18]	106	Perl's staining HIC ($n=93$)	Higher HIC in C282Y heterozygotes ($P<0.05$)	Higher fibrosis score in C282Y heterozygotes ($P<0.05$)	
Tung et al. [19]	198[a]	HIC ($n=198$)	Higher HII[b] in patients with any *HFE* mutation ($P<0.05$)	Odds ratio for advanced fibrosis of 30 ($P<0.05$) by C282Y heterozygosity and of 22 ($P<0.05$) by H63D heterozygosity	Odds ratio calculated for a 15-year duration of HCV-infection
Erhardt et al. [20]	217	Perl's staining	Higher iron scores in C282Y and H63D heterozygotes ($P<0.05$)	Odds ratio of 5.7 ($P<0.05$) for fibrosis and of 6.4 ($P<0.01$) for cirrhosis by C282Y heterozygosity	
Present study	222	Perl's staining	Higher prevalence of C282Y heterozygosity in iron stain positive patients ($P<0.01$)	Odds ratio of 2.5 ($P<0.05$) for advanced fibrosis by C282Y heterozygosity and of 4.8 ($P<0.05$) by C282Y compound heterozygosity	

[a] Patients with compensated liver disease
[b] Hepatic iron index (HIC/age)

HCV-infected patients. Consistent with previous reports [17, 18, 20] heterozygosity for the C282Y mutation in *HFE* was significantly associated with elevated serum ferritin levels and hepatic iron accumulation. The prevalence of the C282Y carrier status in our patients with stainable liver iron was as high as 38.5%. Although we did not determine the hepatic iron concentration in our patients, the high prevalence of the C282Y mutation in HCV-infected patients with stainable liver iron suggests a major role of *HFE* mutations in HCV-associated iron accumulation.

Interestingly, several previously published studies have also demonstrated an association between HCV genotype 1b infection and hepatic iron accumulation [4, 33]. However, the underlying mechanisms are still unclear. The present data indicate that genetic polymorphism in proteins involved in cellular iron uptake may be associated with HCV genotype 1b infection. Among our HCV-infected patients we found a statistical significant association between infection with HCV genotype 1b and C282Y heterozygosity as well as between HCV genotype 1b infection and a combination of C282Y heterozygosity in *HFE* and homozygosity for the S142 allele in *TFR1*. Interestingly, identical interactions between *HFE* and *TFR1* have been described for multiple myeloma [27] as well as breast, colorectal [28], and hepatocellular carcinomas [29]. However, using multivariate logistic regression analysis including sex, age, and fibrosis as variables, these associations did not remain statistically significant. Thus studies including a larger number of patients are required to verify such an interaction.

Another goal of our study was to evaluate the role of *HFE* mutations in HCV-induced liver injury. In our HCV-infected patients we observed a significant association between C282Y heterozygosity and advanced liver fibrosis or cirrhosis (METAVIR score F2–F4). Among HCV-infected patients showing advanced fibrosis or cirrhosis the prevalence of C282Y heterozygosity was 22.6%,

compared with only 8.9% in patients with no or mild fibrosis. Using multivariate logistic regression analysis, the odds ratio for the development of advanced fibrosis or cirrhosis was 2.5 by C282Y heterozygosity and 4.8 by C282Y compound heterozygosity compared with homozygous wild-type HCV-infected patients. These data confirm a previous report describing C282Y heterozygosity as an independent risk factor for the development of HCV-associated hepatic fibrosis in German patients [20]. Erhardt et al. [20] calculated an odds ratio of 5.7 for fibrosis (METAVIR score F1–F4) and an odds ratio of 6.4 for cirrhosis (METAVIR score F4) by C282Y heterozygosity including C282Y/H63D compound heterozygotes. Although our data do not show such a strong association between C282Y heterozygosity and liver fibrosis, they do support the hypothesis that *HFE* mutations play an important role in the progression of HCV-associated liver damage. Such a hypothesis is also supported by a recent study from the United States in which Tung et al. [19] calculated an odds ratio of 30 for the development of bridging fibrosis or cirrhosis over a 15-year duration of HCV infection.

The role of the H63D mutation in the progression of HCV infection is rather controversial. Among our patients we did not find an association between homozygosity or heterozygosity for the H63D mutation and advanced liver fibrosis. This contrasts with recent data from Tung et al. [19] which demonstrated a statistical significant odds ratio of 22 for the development of bridging fibrosis or cirrhosis by heterozygosity for the H63D mutation over a 15-year duration of HCV infection. Erhardt et al. [20] calculated an odds ratio of 2.5 for the development of HCV-associated fibrosis and an odds ratio of 2.9 for the development of HCV-associated cirrhosis by H63D heterozygosity (H63D/WT). These associations did not reach statistical significance [20] and therefore are in good agreement with our data indicating that the H63D mutation plays a minor role in HCV-associated progression of liver fibrosis. The present data also suggest that the frequent S142G polymorphism in *TFR1* is not associated with iron accumulation or disease progression in HCV infection. Similar observations have been recently made in HCV-infected patients from the United States [18].

Taken together, the present data and two recently published studies [19, 20], all including a large number of patients, indicate an important role of C282Y heterozygosity in HCV-associated progression of liver fibrosis. This conclusion contrasts with data from several previously published studies [6, 21, 22, 23, 24], and the discrepancy remains to be elucidated.

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