HEPATITIS

Heterozygous β-globin gene mutations as a risk factor for iron accumulation and liver fibrosis in chronic hepatitis C

Massimo Sartori, Silvano Andorno, Michela Pagliarulo, Cristina Rigamonti, Cristina Bozzola, Patrizia Pergolini, Roberta Rolla, Anna Suno, Renzo Boldorini, Giorgio Bellomo, Emanuele Albano

Gut 2007;**56**:693–698. doi: 10.1136/gut.2006.106641

See end of article for authors' affiliations

Correspondence to: Professor E Albano, Department of Medical Sciences, University "Amedeo Avogadro" of East Piedmont, Via Solaroli 17, 28100 Novara, Italy; albano@med.unipmn.it

Revised 3 October 2006
Accepted 10 October 2006
Published Online First 27 November 2006

Background: Iron accumulation is a well-known risk factor for the progression of chronic hepatitis C (CHC) to fibrosis. However, the profibrogenic role of the genes controlling iron homeostasis is still controversial.
Aim: To evaluate the relative role of haemachromatosis (HFE), ferroportin and β-globin gene mutations in promoting iron accumulation and fibrosis in patients with CHC.
Methods: Genetic analysis was performed together with the assessment of hepatic iron content and histology in 100 consecutive HIV-antibody and hepatitis B surface antigen-negative patients with biopsy-proven CHC.
Results: Among the patients investigated, 12 were heterozygous for various β-globin gene mutations (39[C→T], IVS1.1[G→A], 22 7 bp deletion and IVS1.6[T→C]) and 29 carried HFE (C282Y, H63D and S65C) gene mutations. One further patient was heterozygous for both HFE (H63D) and β-globin (39[C→T]) variants, whereas 58 had the wild-type alleles of both the genes. Hepatic iron concentration (HIC) and hepatic stainable iron were significantly higher ($p<0.05$) in patients with CHC carrying β-globin mutations than in those with HFE mutations or the wild-type alleles. Multivariate analysis confirmed that the presence of β-globin mutations was independently associated with both HIC ($p=0.008$) and hepatic-stainable iron (odds ratio (OR) 6.11; 95% CI 1.56 to 23.92; $p=0.009$). Moderate/severe fibrosis or cirrhosis (Ishak's score >2) was observed in 48 of 100 patients. Logistic regression demonstrated that age (OR 1.05; 95% CI 1.02 to 1.09; $p<0.005$) and β-globin mutations (OR 4.99; 95% CI 1.22 to 20.3; $p=0.025$) were independent predictors of the severity of fibrosis.
Conclusions: Heterozygosis for β-globin mutations is a novel risk factor for both hepatic iron accumulation and the progression to fibrosis in patients with CHC.

Chronic hepatitis C (CHC) is characterised by a high variability in the rate of the disease progression with some patients showing minimal evolution of hepatic injury, and others rapidly developing fibrosis that leads to cirrhosis and hepatocellular carcinoma.[1–3] A number of viral and host-related factors, including older age at infection, alcohol consumption, male gender, coinfections with hepatitis B virus (HBV) or HIV, overweight, and hepatic iron status have been associated with an increased risk of CHC progression.[2 3] Among these, the interaction of hepatitis C virus (HCV) and iron has received increasing attention.[4] Mild iron overload is a common finding in liver biopsy specimens from patients with CHC, and HCV-infected patients with increased hepatic iron content show increased liver fibrosis as compared with patients without iron accumulation.[4 5] Moreover, iron-reducing treatments have been reported to improve liver damage in untreated patients with CHC, as well as to enhance the rate of response to interferon treatment in interferon-resistant subjects.[5] However, the mechanisms responsible for iron accumulation during HCV infection are not fully understood. It has been proposed that necroinflammatory events caused by the virus may lead to alterations in the tissue distribution of the metal.[5] Nonetheless, the role of genetic factors that influence iron trafficking cannot be disregarded. In this context, the broad distribution of polymorphisms of the haemachromatosis (HFE) gene and their importance in causing hereditary HFE[6] has led to the search for a possible role of HFE mutations in the progression of CHC. However, while some studies have shown an association between mutated HFE genotypes and both iron overload and advanced fibrosis or cirrhosis, others have failed to document any significant involvement of HFE mutations in promoting hepatic iron accumulation and CHC evolution.[4 7] Iron trafficking is affected by other genetic defects such as the ferroportin 1 (FPN1) and transferrin receptor 2 (TFR2) mutations, which are involved in non-classic hereditary HFE,[8 9] as well as by the haemoglobin mutations responsible for iron-loading anaemia.[10] Several studies have documented the rapid evolution of hepatic fibrosis in patients with thalassaemia major and HCV infection.[11] Although heterozygous carriers for thalassaemic mutations account for about 1.67% of the world population,[10] the possible contribution of thalassaemic traits in favouring iron accumulation in CHC has not been characterised. The main purpose of this study was to explore the possible associations between different mutations in the β-globin, HFE, FPN1 and TFR2 genes, and both hepatic iron content and the severity of liver fibrosis among Italian patients with CHC.

METHODS

Patient recruitment

For this study, 100 consecutive HIV antibody- and hepatitis B surface antigen-negative patients with CHC (54 men and 46 women; mean age 52 years) were recruited from January 2002 to February 2004. All of them had untreated CHC, defined as having a liver biopsy assessed by a pathologist, a second-generation positive enzyme-linked immunosorbent assay and the presence of HCV-RNA in the serum evaluated by nested PCR. The criteria of exclusion were current drug addiction, hepatitis B surface antigen or anti-HIV positivity, every other known liver disease, and present or previous antiviral treatment. All subjects gave informed consent to the analysis. The

Abbreviations: CHC, chronic hepatitis C; FPN1, ferroportin 1; HCV, hepatitis C virus; HFE, haemachromatosis; HIC, hepatic iron concentration; TFR2, transferrin receptor 2

study was planned according to the guidelines of the local ethical committee in conformity with the 1975 Declaration of Helsinki. Anti-HCV status was assessed by second-generation enzyme-linked immunosorbent assay (Ortho Diagnostic System, Milan, Italy). HCV-RNA was detected in the serum by the Amplicor HCV Kit (Roche Diagnostic System, Branchburg, New Jersey, USA) following the manufacturer's instructions. The lifetime mean daily alcohol consumption was assessed at the time of admission with a standardised questionnaire, presented as part of a survey on life habits. Alcohol intake >50 g/day for men and >20 g/day for women in the 5 years that preceded the hospital admission were considered.

Mutation analysis

DNA was extracted from peripheral blood mononucleated cells using QIAamp DNA extraction kit (QIAGEN, Valencia, California, USA) according to the manufacturer's instructions.

The polymorphisms of the β-globin, HFE, TFR2 and FPN1 genes were assessed using the β-Globin StripAssay and Haemochromatosis StripAssay A (ViennaLab Labordiagnistika GmbH, Vienna, Austria), consisting of PCR amplification of the sequences of interest using biotinylated primers, followed by the hybridisation of the amplification products to a test strip containing immobilised allele-specific oligonucleotide probes. Bound biotinylated sequences are detected using streptavidin–alkaline phosphatase and colour substrates. The following 22 mutations of the β-globin gene were investigated: –87 [C→G], –30[T→A], codon 5 [−CT], Hb C, Hb S, codon 6 [−A], codon 8 [−AA], codon 8/9 [+G], codon 22 [7 bp del], codon 30 [G→C], IVS1.1 [G→A], IVS1.2 [T→A], IVS1.5 [G→C], IVS1.6 [T→C], IVS1.110 [G→A], IVS1.116 [T→G], IVS1–25 [25 bp del], codon 36/37 [−T], codon 39 [C→T], codon 44 [−C], IVS2.1 [G→A], IVS2.745 [C→G]. The HFE gene was analysed for 11 mutations as follows: V53M, V59M, H63D, S65C, Q127H, P160delC, E168Q, E168X, W169X, C282Y and Q283P9. The presence of E60X, M172K, Y250X and AVAQ594-597del mutations of TFR2 and N144H, V162del of the FPN1 genes was also evaluated by the same procedure. The accuracy of the assays was confirmed by sequencing randomly selected DNAs.

Liver biopsies

Liver biopsy was performed at the time of the admission using a modified Menghini procedure. On a portion of each sample, hepatic iron concentration (HIC) was measured by atomic absorption spectroscopy and expressed as μmol/g dry weight. The cut-off value for HIC was fixed at 25 μmol/g. Hepatic iron index was calculated as reported previously.[12] The remainder of the sample was immediately fixed in formalin and embedded in paraffin wax. Sample sections of thickness 5 μm were stained with haematoxylin and eosin, Masson's trichrome and periodic acid-Schiff after diastase digestion, as well as with the Gomori's method for reticulin and the Perls's method for iron staining. The grading and the staging of chronic hepatitis were scored according to Ishak's criteria.[13] Histological assessment of hepatic iron stores was performed using the score system proposed by Searle *et al.*[14] All histological evaluations were performed double-blind by two experienced pathologists. In the case of discordant opinions, the two examiners analysed the discrepancies to reach a consensus.

Statistical analysis

Stata Statistical Software V.9.0 was used in all the statistical analyses. Each variable predictive of or associated with the presence of hepatic fibrosis, HIC and hepatic stainable iron was analysed with univariate linear regression (when indicated) and/or with univariate logistic regression. The variables selected by every univariate analysis were entered into linear and/or logistic regression models using a forward stepwise elimination algorithm (terms with $p>0.05$ were eligible for removal). The analysis of variance model was used to compare the continuous variables among groups, and multiple comparison tests were performed with the Bonferroni's correction. The Kruskal–Wallis model and the Wilcoxon rank-sum test were used to compare discrete variables.

RESULTS

Table 1 summarises the demographic, clinical and laboratory characteristics of the patients with CHC included in this study. The probable source, the duration of the infection and the age at infection were available for 41 patients, who received blood transfusions before 1990 (31 patients) or shared syringes while using illicit drugs (10 patients). Genetic characterisation of all the 100 patients with CHC demonstrated that 12 were heterozygotic for one of the different β-globin gene mutations (codon 39 C→T in seven cases, codon IVS 1.1 G→A in three cases, codon 22 7 bp deletion in one case and codon IVS 1.6 T→C in one case) affecting haemoglobin synthesis, and that 29 carried HFE gene mutations (heterozygotic C282Y in three cases, homozygotic H63D in three cases, heterozygotic H63D in 22 cases and S65C heterozygous in one case). One further patient was heterozygotic for both HFE (H63D) and β-globin (codon 39 C→T) variants. In all, 58 patients had the wild-type alleles of both the genes (table 2). None of the patients carried mutations of the FPN1 and TFR2 genes (table 2).

As expected, haemoglobin was lower in patients with CHC and mutated β-globin (11.8 (1.95) g/dl; 95% CI 10.6 to 13.0; $p<0.001$) than in those with HFE variants (14.4 (1.5) g/dl; 95% CI 13.8 to 15.0) or the wild-type alleles (14.4 (1.4) g/dl; 95% CI 14.0 to 14.8). Intrahepatic accumulation of iron, as estimated by both HIC and iron staining by the Perls's method, was evident in, respectively, 22 of 96 (23%) and 40 of 98 (41%) subjects investigated. HIC and hepatic iron staining were significantly higher ($p<0.05$ and $p<0.01$, respectively) in patients with CHC with β-globin mutations than in those with HFE mutations or the wild-type alleles (table 3). The carriers of β-globin variants also had higher serum ferritin levels ($p<0.05$) than the patients with mutant HFE (table 3). Transferrin saturation was increased in patients with CHC with β-globin mutations as compared with those with the wild-type alleles (table 3). No difference was appreciable in the serum iron content between the three groups (table 3).

In both univariate and multivariate analyses, the presence of β-globin mutations was the only variable found to be associated ($p = 0.008$) with HIC (table 4). Male gender (odds ratio (OR) 2.55; 95% CI 1.10 to 5.92; $p = 0.029$), necroinflammatory grading (OR 1.25; 95% CI 1.02 to 1.53; $p = 0.029$) and mutated β-globin alleles (OR 6.11; 95% CI 1.56 to 23.92; $p = 0.009$) were also associated with the detection of hepatic stainable iron (Searle's score >0) (table 4). Nonetheless, following multivariate logistic regression analysis, the heterozygosis for the different β-globin mutations was the only risk factor (OR 6.11; 95% CI 1.56 to 23.92; $p = 0.009$) for the histological detection of iron deposition.

Histology revealed the presence of moderate/severe liver fibrosis (staging >2) in 48 of 100 patients with CHC and cirrhosis (staging >4) in 14 patients (table 1). The scores for both histological grading and staging were significantly ($p<0.05$) higher in the patients with β-globin mutations as compared with those with the HFE variants or the wild-type alleles (table 3). Logistic regression analysis taking the presence of moderate/severe fibrosis (staging score >2) as dependent variable revealed that age (OR 1.05; 95% CI 1.02 to 1.09; $p<0.005$) and β-globin mutations (OR 4.99; 95% CI 1.22 to

Table 1 Epidemiological, clinical and laboratory characteristics of 100 patients with chronic hepatitis C

Variable	Number	Mean (SD)	Range
Age (years)		54.2 (13.9)	19–74
Gender (male/female)	54/46		
Drug addiction (yes/no)	10/90		
Previous transfusion (yes/no)	31/69		
Alcohol intake (⩾50 g/day for men and ⩾20 g for women) (yes/no)	7/93		
Duration of infection (years)*		22 (8.0)	3–45
Age at infection (years)*		23.9 (11.4)	1–55
HCV genotype (1/non-1)	50/50		
Serum iron (μmol/l; 11–32 μmol/l)		24.3 (9.5)	6.3–56.1
Transferrin saturation (%; 20–50)		41.9 (17.9)	9–97
Serum ferritin (ng/ml; 5–365 ng/ml)		320 (363)	6–2900
HIC (μmol/g dry tissue; <25 μmol/g)†		22.1 (26.9)	2.2–245.5
Iron index (HIC/age; <1.9)†		0.5 (0.7)	0.1–5.3
Hepatic iron grading (Searle's score system; 0)		0‡	0–4
Platelets (elements/μl; 150–450 elements/μl)		193 (67)	57–433
Aspartate aminotransferase (U/l; 0–40 U/l)		69 (45)	20–279
Alanine aminotransferase (U/l; 0–40 U/l)		102 (68)	21–384
γ-Glutamyl transpeptidase (U/l; 0–50 U/l)		57 (43)	7–197
Alkaline phosphatase (U/l; 90–360 U/l)		159 (59)	45–361
Bilirubin (μmol/l; 2–18 μmol/l)		13.7 (8.5)	1.7–39.3
Albumin (g/dl; 4–6 g/dl)		3.9 (0.3)	2.7–5.4
γ-Globulins (g/dl; 0.6–1.8 g/dl)		1.4 (0.3)	0.6–2.6
Prothrombin time (%; 70–110)		93.8 (8.7)	67–109
Histological grading (Ishak's score)		3§	1–8
Histological staging (Ishak's score)		2§	0–6
Significant fibrosis (Ishak's score >2) (yes/no)	48/52		
Histological cirrhosis (Ishak's score >4) (yes/no)	14/86		
Any mutation of HFE gene (yes/no)‡	30/70		
Any mutation of β-globin gene (yes/no)‡	13/87		
Any mutation of HFE gene or β-globin gene (yes/no)‡	42/58		

HCV, hepatitis C virus; HFE, haemachromatosis; HIC, hepatic iron concentration.
*Data were available for 41 of 100 patients.
†Data were available for 97 of 100 patients.
‡One patient had both an HFE and a β-globin gene mutation.
§Values are medians.

Table 2 Mutations in the haemachromatosis, ferroportin 1, transferrin receptor 2 and β-globin genes detected by polymerase chain reaction and reverse-hybridisation assays in 100 patients with chronic hepatitis C

	Observed mutations (n)	β-Globin gene	Observed mutations (n)
HFE gene			
C282Y	3	−87 [C→G]	0
H63D	26	−30 [T→A]	0
E168X	0	Codon 5 [−CT]	0
W169X	0	Haemoglobin C	0
S65C	1	Haemoglobin S	0
V53M	0	Codon 6 [−A]	0
V59M	0	Codon 8 [−AA]	0
Q283P	0	Codon 8/9 [+G]	0
E168Q	0	Codon 22 [7bp del]	1
Q127H	0	Codon 30 [G→C]	0
P160delC	0	IVS 1.1 [G→A]	4
		IVS 1.2 [T→A]	0
TFR2 gene		IVS 1.5 [G→C]	0
Y250X	0	IVS 1.6 [T→C]	0
E60X	0	IVS 1.110 [G→A]	0
M172K	0	IVS 1.116 [T→G]	1
AVAQ594–597del	0	IVS 1–25 [25bp del]	0
		Codon 36/37 [−T]	0
FPN1 gene		Codon 39 [C→T]	7
N144H	0	Codon 44 [−C]	0
V162 del	0	IVS 2.1 [G→A]	0
		IVS 2.745 [C→G]	0

FPN1, ferroportin; HFE, haemachromatosis; TFR2, transferrin receptor 2.

20.3; p = 0.025) were the only independent predictors of moderate/severe fibrosis or cirrhosis in the patients investigated (table 5). Similar results (age OR 1.04; 95% CI 1.01 to 1.09; p = 0.008; β-globin mutations OR 5.0; 95% CI 1.24 to 20.2; p = 0.024) were obtained after correction for the possible confounding action of the high alcohol intake in seven of the patients investigated.

DISCUSSION

In 1992, Di Bisceglie *et al*[15] first reported that a high proportion of patients with CHC had abnormal serum iron values. Such an observation has been confirmed by subsequent studies showing a correlation between the extent of hepatic stainable iron and the severity of hepatitis.[5] Moreover, we have reported that menstruating women experienced a milder CHC than men of the same age, in relation to the lower HIC due to blood losses.[16] Consistently, a number of studies have shown that lowering the iron stores by phlebotomy or other iron-reducing treatments improves the evolution of CHC and enhances the response to interferon treatments.[4 5] The mechanisms responsible for the worsening of HCV infection by iron have not been characterised in detail. Recent observations suggest that iron might enhance HCV replication by stimulating the expression of translation initiation factor 3.[17] However, other evidence points to the interference of iron with the virus replication processes.[18] Nonetheless, the notions that HCV proteins stimulate the formation of reactive oxygen species within the hepatocytes and that iron exacerbates oxidative damage suggest that iron accumulation might amplify oxidative stress-mediated events, leading to both hepatocellular damage and fibrogenic

Table 3 Iron status and liver histology in patients with chronic hepatitis C and different genetic background*

	Patients with any β-globin gene mutations	Patients with any HFE gene mutations	Patients with wild-type alleles†
Number of patients‡	13	30	58
Serum iron (μmol/l)	26.8 (8.3)	25.9 (9.3)	23.1 (9.6)
Transferrin saturation (%)	53.9 (25.0)§	41.1 (12.4)	39.8 (17.6)
Serum ferritin (ng/ml)	551 (777)¶	255 (215)	304 (258)
HIC (μmol/g)**	42.2 (68.5)§¶	19.1 (12.7)	19.5 (14.8)
Hepatic iron grading (Searle's score)	2 (0–4)††‡‡	0 (0–3)	0 (0–3)
Histological grading (Ishak's score)	4 (2–8)‡‡§§	3 (1–8)	3 (1–8)
Histological staging (Ishak's score)	3 (2–6)¶¶§§	2 (0–6)	2 (0–6)

HFE, haemachromatosis; HIC, hepatic iron concentration.
*Values are mean (SD). Values of hepatic iron grading, histological grading and staging are medians with ranges in brackets.
†The patients with the wild-type alleles do not have any β-globin or HFE mutations.
‡Total number of patients is 101 because one patient had both a β-globin and an HFE gene mutation.
§$p<0.05$ versus patients with the wild-type alleles (with Bonferroni's correction).
¶$p<0.05$ versus patients with any HFE gene mutations (with Bonferroni's correction).
**HIC was evaluated in 11 patients with β-globin gene mutation, 30 patients with HFE gene mutation(s) and 56 patients with the wild-type alleles.
††$p<0.01$ versus patients with the wild-type alleles (Wilcoxon rank sum test).
‡‡$p<0.01$ versus patients with any HFE gene mutations (Wilcoxon rank sum test).
§§$p<0.05$ versus patients with the wild-type alleles (Wilcoxon rank sum test).
¶¶$p<0.05$ versus patients with any HFE gene mutations (Wilcoxon rank sum test).

processes.[19] Supporting this view, Furutani *et al*[20] have recently shown that mild iron accumulation in the liver of transgenic mice overexpressing HCV polyprotein enhances lipid peroxidation and promotes the development of hepatocellular carcinomas.

At present, the mechanisms responsible for hepatic iron deposition during HCV infection are not fully understood. It has been proposed that necroinflammation sustained by the virus along with genetic factors that modify iron trafficking might lead to the alterations in iron homeostasis.[5] In humans, the only known regulated step in iron trafficking is the intestinal absorption.[9] Hepcidin, a 25 amino acid peptide synthesised in the liver, plays a key role in such a process by inhibiting iron efflux from the enterocytes as well as the recycling of the metal by the macrophages.[21] Recently, Aoki *et al*[22] have reported that, in patients with CHC, liver hepcidin mRNA expression is not influenced by necroinflammation and fibrosis and increases in relation with hepatic iron concentration. This suggests that iron stores regulate hepcidin expression in patients with CHC, and that iron accumulation is not due to inappropriate hepcidin expression. However, other hepatic proteins essential for the normal iron homeostasis, including HFE, TFR2 and haemojuvelin, have been proposed to modulate the expression of hepcidin.[9 21] Indeed, hepcidin production is decreased in patients with hereditary HFE and in HFE-knockout mice.[9] A number of studies have addressed the role of the common HFE mutations in favouring hepatic iron accumulation during CHC, obtaining conflicting results.[4 7] These discrepancies might be due to the failure to correct for confounding factors.[7] Moreover, as pointed out by Tung *et al*,[23] the association between HFE mutations and the severity of both iron accumulation and fibrosis is more evident in patients with compensated disease after controlling for the duration of infection, and disappears in patients with end-stage liver disease. Although the patients included in the present study all have compensated CHC, we failed to see a contribution of HFE mutations in causing hepatic

Table 4 Univariate logistic and linear regression analyses of the putative predictors of hepatic iron accumulation in patients with chronic hepatitis C

	Hepatic stainable iron (grading >0 according to Searle's score)		HIC (μmol/g)	
Univariate predictors	**Odds ratio (95% CI*)**	**p Value**	**95% CI***	**p Value**
Age (years)	1.006 (0.97 to 1.03)	0.718	−0.52 to −0.30	0.598
Gender†	2.556 (1.10 to 5.92)	0.029	−3.22 to −18.4	0.166
Drug addiction	1.514 (0.41 to 5.61)	0.535	−21.6 to −17.9	0.853
Transfusion	0.988 (0.96 to 1.02)	0.445	−0.24 to −0.48	0.507
Alcohol intake‡	0.557 (0.10 to 3.02)	0.499	−22.2 to −19.8	0.912
Duration of infection (years)§	1.035 (0.95 to 1.12)	0.416	−2.32 to −0.82	0.343
Age at infection (years)§	1.006 (0.95 to 1.06)	0.811	−0.57 to −1.45	0.382
HCV genotype (1 versus non-1)¶	1.269 (0.57 to 2.84)	0.563	−20.7 to −0.74	0.068
Grading†	1.253 (1.02 to 1.53)	0.029	−1.33 to −4.04	0.321
Staging	1.239 (0.96 to 1.59)	0.098	−2.50 to −4.39	0.588
Any HFE gene mutation	0.633 (0.25 to 1.55)	0.318	−16.1 to −7.41	0.464
Any β-globin gene mutation†	6.111 (1.56 to 23.9)	0.009	6.08 to −39.2	0.008
Any HFE or β-globin gene mutation	1.376 (0.61 to 3.10)	0.441	−4.79 to −17.3	0.264

HCV, hepatitis C virus; HFE, haemachromatosis; HIC, hepatic iron concentration.
*CI denotes confidence interval respectively of the odds ratio and of the regression slope.
†These predictors were selected to enter in the multivariate logistic model (see Results).
‡Alcohol intake ⩾50 g/day for men or ⩾20 g/day for women.
§Data were available for 41 of 100 patients.
¶Data were available for 97 of 100 patients.

Table 5 Univariate and multivariate logistic regression analysis of the putative predictors of moderate/severe hepatic fibrosis in patients with chronic hepatitis C

	Odds ratio (95% CI)	p Value
Univariate analysis		
Age (years)*	1.047 (1.01 to 1.08)	0.006
Gender	1.013 (0.46 to 2.22)	0.974
Drug addiction	0.697 (0.18 to 2.64)	0.595
Transfusion	0.992 (0.96 to 1.02)	0.541
Alcohol intake (⩾50 g/day for men and ⩾20 g for women)	7.286 (0.84 to 62.9)	0.071
Duration of infection (years)†	1.073 (0.98 to 1.18)	0.142
Age at infection (years)†	1.048 (0.99 to 1.11)	0.128
HCV genotype (1 versus non-1)‡	1.268 (0.58 to 2.78)	0.554
Serum iron (μmol/l)	1.002 (0.99 to 1.01)	0.547
Transferrin saturation (%)	1.007 (0.98 to 1.03)	0.545
Serum ferritin (ng/ml)	1.000 (0.99 to 1.00)	0.478
HIC (μmol/g)	1.001 (0.98 to 1.01)	0.913
Hepatic stainable iron (Searle's score)	1.577 (0.98 to 2.53)	0.059
Any HFE gene mutation	0.926 (0.39 to 2.18)	0.861
Any β-globin gene mutation*	4.298 (1.10 to 6.70)	0.035
Any HFE or β-globin gene mutation	1.598 (0.72 to 3.55)	0.251
Multivariate analysis		
Age (years)	1.054 (1.01 to 1.09)	0.005
Any β-globin gene mutation	4.992 (1.22 to 20.3)	0.025

HCV, hepatitis C virus; HFE, haemachromatosis; HIC, hepatic iron concentration.
*These predictors were selected to enter in the multivariate logistic model (see Results section).
†Data were available for 41 of 100 patients.
‡Data were available for 97 of 100 patients.

iron accumulation and disease progression. This might be ascribed to the predominance of the HFE H63D variant among our patients, which affects iron homeostasis in CHC to a lower extent than the C282Y variant.[24 25] Moreover, the presence of other, still poorly characterised, factors that interfere with the phenotypical expression of mutated HFE genes cannot be excluded.[26]

Thalassaemia represents a common cause of iron overload in many countries of the Mediterranean, Middle East and South Asia, and its frequency is rapidly increasing in the US.[27] The combination of HCV infection and iron overload is a well-established risk factor for hepatic cirrhosis and hepatocellular carcinoma among patients with β-thalassaemia.[28 29] However, in spite of the high frequency of thalassaemic trait carriers worldwide, the contribution of heterozygotic thalassaemic variants to the evolution of hepatitis C has so far been neglected. Our observation that patients with CHC heterozygotic for different β-globin mutations have a 5–6-fold increased risk of both iron overload and extensive fibrosis/cirrhosis adds new emphasis to the role of the haemoglobin defects in the progression of HCV infection. It is noteworthy that the frequency of β-thalassaemic traits among our patients with CHC is higher (13%) compared with that in the general population from the same area of north Italy (about 4%),[30] probably because of a clustering of the mutant β-globin alleles among the subjects with active hepatitis C. In patients with β-thalassaemia, major ineffective erythropoiesis and anaemia are important stimuli for intestinal iron absorption, despite a massive iron overload.[31] Consistently, urinary hepcidin levels are very low or undetectable in patients with thalassaemia major or intermedia.[32] A low hepatic expression of hepcidin mRNA is also detectable in the mouse models of the different forms of thalassaemia.[33] Impaired erythropoiesis and increased iron absorption are evident in the carriers of β-thalassaemic traits,[34] suggesting a decrease in hepcidin production. Thus, it is possible to speculate that, even though HCV infection by itself does not influence hepatic hepcidin synthesis, patients with CHC heterozygotic for β-globin mutations experience hepatic iron accumulation because of the predominance of the signals from the erythroid compartments over those coming from the iron stores in down-modulating hepcidin production. Accordingly, the incubation of HepG2 cells with the sera from patients with thalassaemia decreases hepcidin mRNA expression.[35]

In conclusion, our study identifies the heterozygosis for β-globin gene mutations as a new independent risk factor for iron accumulation and the progression of liver fibrosis in patients with CHC, and suggests the presence of thalassaemic traits as a possible contributor to the alterations in iron homeostasis associated with chronic liver diseases.

ACKNOWLEDGEMENTS

This work has been supported by grants from the University of East Piedmont, Regional Government of Piedmont, and from the Italian Ministry for University and Scientific and Technological Research (Research Program: 200401213 Epatopatia steatosica non alcolica; studio dei fattori metabolici, genetici e virologici responsabili del danno epatico e della sua evoluzione).

Authors' affiliations

Massimo Sartori, Silvano Andorno, Michela Pagliarulo, Gastroenterology Units, Ospedale Maggiore della Carità, Novara, Italy
Cristina Rigamonti, Cristina Bozzola, Roberta Rolla, Renzo Boldorini, Giorgio Bellomo, Emanuele Albano, Department of Medical Sciences, University ''Amedeo Avogadro'' of East Piedmont, Novara, Italy
Patrizia Pergolini, Anna Suno, Clinical Chemistry Units, Ospedale Maggiore della Carità, Novara, Italy

Competing interests: None.

REFERENCES

1 **Patel K**, Muir AJ, McHutchison JG. Diagnosis and treatment of chronic hepatitis C infection. *BMJ* 2006;**332**:1013–17.
2 **Feld JJ**, Liang TJ. Hepatitis C—identifying patients with progressive liver injury. *Hepatology* 2006;**43**:S194–206.
3 **Massard J**, Ratziu V, Thabut D, *et al*. Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol* 2006;**44**:S19–24.
4 **Eisenbach C**, Gehrke SG, Stremmel W. Iron, the HFE gene, and hepatitis C. *Clin Liver Dis* 2004;**8**:775–85.
5 **Alla V**, Bonkovsky HL. Iron in nonhemochromatotic liver disorders. *Semin Liver Dis* 2005;**25**:461–72.
6 **Beutler E**. Hemochromatosis: genetics and pathophysiology. *Annu Rev Med* 2006;**57**:331–47.
7 **Pietrangelo A**. Hemochromatosis gene modifies course of hepatitis C viral infection. *Gastroenterology* 2003;**124**:1509–23.
8 **Deicher R**, Hörl WH. New insights into the regulation of iron homeostasis. *Eur J Clin Invest* 2006;**36**:301–9.
9 **Pietrangelo A**. Molecular insights into the pathogenesis of hereditary haemochromatosis. *Gut* 2006;**55**:564–8.
10 **Angastiniotis M**, Modell B. Global epidemiology of hemoglobin disorders. *Ann NY Acad Sci* 1997;**336**:1298–301.
11 **Vento S**, Caianello F, Cesario F. Infection and thalassemia. *Lancet Infect Dis* 2006;**6**:226–33.
12 **Sartori M**, Andorno S, La Terra G, *et al*. Evaluation of iron status in patients with chronic hepatitis C. *Ital J Gastroenterol Hepatol* 1998;**30**:396–401.
13 **Ishak K**, Baptista A, Bianchi L, *et al*. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;**22**:696–9.
14 **Searle JW**, Kerr JFR, Halliday JW, *et al*. Iron storage diseases. In: MacSween RNM, Antony PP, Scheuer PJ, eds. *Pathology of the liver*, 2nd edn. Edinburgh: Churchill Livingston, 1987.
15 **Di Bisceglie AM**, Axiotis CA, Hoffnagle JH, *et al*. Measurements of iron status in patients with chronic hepatitis. *Gastroenterology* 1992;**102**:2108–13.
16 **Sartori M**, Andorno S, Rigamonti C, *et al*. Chronic hepatitis C is mild in menstruating women. *J Gastroenterol Hepatol* 2000;**15**:1411–17.
17 **Theurl I**, Zoller H, Obrist P, *et al*. Iron regulates hepatitis C virus translation via stimulation of expression of translation initiator factor 3. *J Infect Dis* 2004;**190**:819–25.
18 **Fillebeen C**, Rivas-Estilla AM, Bisaillon M, *et al*. Iron inactivates the RNA polymerase NS5B and suppress subgenomic replication of hepatitis C virus. *J Biol Chem* 2005;**280**:9049–57.
19 **Choi J**, Ou JH. Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus. *Am J Physiol* 2006;**290**:G847–51.

20 **Furutani T**, Hino K, Okuda M, *et al*. Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. *Gastroenterology* 2006;**130**:2087–98.
21 **Ganz T**, Nemeth E. Iron Imports. IV. Hepcidin and the regulation of body iron metabolism. *Am J Physiol* 2006;**290**:G199–203.
22 **Aoki CA**, Rossaro L, Ramsamooj R, *et al*. Liver hepcidin mRNA correlates with iron stores, but not inflammation, in patients with chronic hepatitis C. *J Clin Gastroenterol* 2005;**39**:71–4.
23 **Tung BY**, Edmond MJ, Bronner MP, *et al*. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. *Gastroenterology* 2003;**124**:318–26.
24 **Bonkovsky HL**, Troy N, McNeal K, *et al*. Iron and *HFE* or *TfR1* mutations as comorbid factors for development and progression of chronic hepatitis C. *J Hepatol* 2002;**37**:848–54.
25 **Erhardt A**, Maschner-Olberg A, Mellenthin C, *et al*. HFE mutations and chronic hepatitis C: H63D and C282Y heterozygosity are independent risk factors for liver fibrosis and cirrhosis. *J Hepatol* 2003;**38**:335–42.
26 **Beutler E**, Felitti VJ, Koziol JA, *et al*. Penetrance of 845G→A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet* 2002;**359**:211–18.
27 **Cohen AR**, Galanello R, Pennell DJ, *et al*. Thalassemia. *Hematology* 2004:14–34.
28 **Angelucci E**, Muretto P, Nicolucci A, *et al*. Effects of iron overload and hepatitis C virus positivity in determining progression of liver fibrosis in thalassemia following bone marrow transplantation. *Blood* 2002;**100**:17–21.
29 **Borgna-Pignatti C**, Vergine G, Lombardo T, *et al*. Hepatocellular carcinoma in the thalassaemia syndromes. *Br J Haematol* 2004;**124**:114–17.
30 **Silvestroni E**, Bianco I. Screening for microcytemia in Italy: analysis of data collected in the past 30 years. *Am J Hum Gen* 1975;**27**:198–212.
31 **Rund D**, Rachmilewitz E. β-Thalassemia. *New Engl J Med* 2005;**353**:1135–46.
32 **Papanikolaou G**, Tzillianos M, Christakis JI, *et al*. Hepcidin in iron overload disorders. *Blood* 2005;**105**:4103–5.
33 **Weizer-Stern O**, Adamsky K, Amariglio N, *et al*. mRNA expression of iron regulatory genes in beta-thalassemia intermedia and beta-thalassemia major mouse models. *Am J Hematol* 2006;**8**:479–83.
34 **Pippard MJ**, Wainscoat JS. Erythrokinetics and iron status in heterozygous beta thalassaemia, and the effect of interaction with alpha thalassaemia. *Br J Haematol* 1987;**66**:123–7.
35 **Rachmilewitz EA**, Weizer-Stern O, Adamsky K, *et al*. Role of iron in inducing oxidative stress in thalassemia: can it be prevented by inhibition of absorption and by antioxidants? *Ann NY Acad Sci* 2005;**1054**:118–23.

doi: 10.1136/gut.2006.096123a

Answer

From the question on page 644

Food debris was drained out from the chest tube 36 h after admission. Distal oesophageal rupture was confirmed by an oesophagogram (fig 1), which showed a massive leakage (arrow) of barium to the mediastinum and left pleural space (arrowheads). The patient was diagnosed with Boerhaave's syndrome complicated with secondary pleural effusion.

Boerhaave's syndrome is an uncommon clinical entity, which is defined as spontaneous oesophageal rupture excluding perforations resulting from iatrogenic instrumentation or foreign bodies. Classic presentation of Mackler's triads, vomiting, chest pain and subcutaneous emphysema are not common. Atypical presentations include asymptomatic pleural effusion, dyspnoea secondary to pneumothorax or hydrothorax, or haematemesis. The presence of pneumomediastinum or pneumothorax offers important clues for diagnosis, and should be carefully looked for on the chest radiograph. The most typical chest *x* ray finding is left-sided hydropneumothorax, which is secondary to left posterolateral oesophageal rupture due to inherited anatomical weakness. Primary surgical repair of the perforation is the definite treatment. The best results are achieved when operative repair is within 12 h of rupture. Rising mortality can be expected if surgery is delayed owing to increasing frequency of complications.

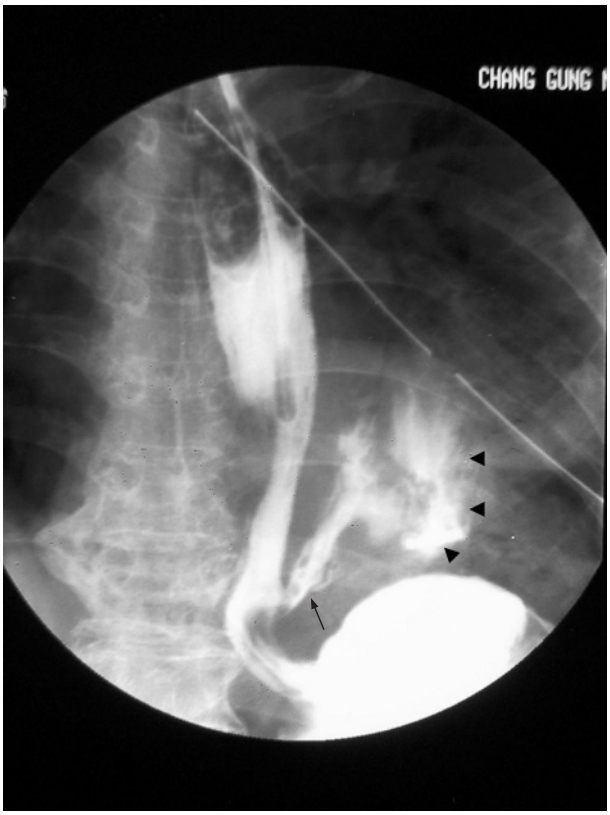

Figure 1 Oesophagogram showing a massive leakage (arrow) of barium to the mediastinum and left pleural space (arrowheads).