Lack of association between HFE gene mutations and hepatocellular carcinoma in patients with cirrhosis

V Boige, L Castéra, N de Roux, N Ganne-Carrié, B Ducot, G Pelletier, M Beaugrandid, C Buffet

Liver cirrhosis is a major aetiological factor for the occurrence of hepatocellular carcinoma (HCC). The annual incidence of HCC in patients with liver cirrhosis is 3–5%. However, not all patients with cirrhosis develop HCC. Thus identification of high risk patients for HCC is important to increase the rate of early detection by screening programmes and subsequently to try to improve the impact of radical curative therapies on survival. Several risk factors for developing HCC have been identified in Western patients with cirrhosis, including male sex, age above 50 years, persistently raised serum α fetoprotein levels, severity of cirrhosis (large oesophageal varices and abnormal prothrombin time), and anti-hepatitis C antibodies. Patients with genetic haemochromatosis (GH) are also at high risk for developing HCC, even in the absence of cirrhosis or despite complete depletion of tissue iron. Two missense mutations of the HFE gene have been associated with 60–90% of all cases of GH. Eighty two to 100% of patients with GH are homozygous for the first and major mutation termed C282Y and 4% are compound heterozygotes for the C282Y mutation and the second and minor mutation termed H63D.

An increased prevalence of the HFE C282Y mutation has recently been reported in patients with HCC that developed in the non-cirrhotic liver with mild iron overload, suggesting a possible involvement of the heterozygous state for the C282Y mutation in hepatocarcinogenesis. Such a putative role for H63D remains elusive as this mutation in the absence of C282Y has not been associated with significant iron overload or HCC thus far. This prompted us to prospectively assess the prevalence of C282Y and H63D mutations in cirrhotic patients with or without HCC.

Aim: To assess the prevalence of HFE gene mutations in cirrhotic patients with and without HCC.

Patients and methods: A total of 133 consecutive cirrhotic patients with HCC were prospectively studied for the presence of C282Y and H63D mutations. The control group consisted of 100 cirrhotic patients without HCC. We used restriction enzyme digestion of polymerase chain reaction amplified genomic DNA for determination of HFE genotypes. Iron loading was assessed on non-tumoral liver biopsy samples from 89 patients with HCC and 73 patients without HCC.

Results: The prevalence of C282Y heterozygotes was similar in patients with and without HCC (5% vs 4%, respectively; p=0.65) and did not differ from that expected in the general population. None of the HCC patients was found to be homozygous for C282Y or H63D, nor compound heterozygous. The prevalence of H63D heterozygotes was similar in patients with and without HCC (31% vs 38%, respectively; p=0.25). No relation was detected between HFE genotypes and hepatic iron loading in patients with or without HCC.

Conclusion: C282Y and H63D mutations do not appear to be associated with an increased risk of HCC in patients with cirrhosis.
Liver iron loading was assessed in a semiquantitative fashion using Perl’s Prussian blue staining on 162 available non-tumoral liver biopsy samples (89 with and 73 without HCC) as follows: 0, no staining; 1, minimal to moderate iron overload (<50% stained hepatocytes); and 2, massive iron overload (>50% stained hepatocytes).

**Statistics**

The two groups of patients (with or without HCC) were compared according to the presence or the absence of HCC, as follows: 0, no staining; 1, minimal to moderate iron overload (<50% stained hepatocytes); and 2, massive iron overload (>50% stained hepatocytes).

**RESULTS**

Patient characteristics and the distribution of HFE genotypes, according to the presence or the absence of HCC, are given in table 1. Except for age and sex, the two groups were comparable, particularly for aetiology of cirrhosis.

The C282Y mutation was present on 2.6% of chromosomes from patients with HCC compared with 3.0% of chromosomes from patients without HCC (p=0.44). The prevalence of C282Y heterozygotes was similar in patients with and without HCC (5% vs 4%, respectively; p=0.65) and did not differ from that expected in the normal French population (3.6%).

The frequency of the H63D mutation was 15.4% of chromosomes from patients with HCC compared with 20.5% in those without (p=0.12). The prevalence of H63D heterozygotes was similar in patients with and without HCC (31% vs 38%, respectively; p=0.25) and slightly higher than that of 23.7% expected in the normal French population. None of the HCC patients was found to be a C282Y homozygote, H63D homozygote, or compound heterozygote.

Overall, no significant difference for the prevalence of C282Y and H63D mutations was observed between the two groups, even after adjustment for age and sex. When patients were studied according to aetiology of cirrhosis, no significant difference for the prevalence of the C282Y and H63D mutations was observed between the two groups of patients (table 2). Similarly, when considering patients with and without HCC together, the distribution of HFE genotypes did not differ according to the aetiology of cirrhosis.

Patients with available non-tumoral liver tissue samples did not differ from those without for most characteristics (age, sex, aetiology of cirrhosis, HFE genotypes). Hepatic iron loading did not differ between patients with and without HCC (p=0.8). Finally, no significant correlation between HFE genotype and hepatic iron loading was observed in patients with or without HCC (table 3).

**DISCUSSION**

The main result of this large prospective multicentre study was that the prevalence of C282Y and H63D HFE gene mutations did not differ between cirrhotic patients with and without HCC.

Several studies have previously suggested that the prevalence of HFE gene mutations was higher in cirrhotic patients with HCC compared with those without HCC (0.5% vs 0.3%, respectively; p=0.0003). The prevalence of HFE gene mutations was higher in cirrhotic patients without HCC.

**Table 1** Clinical characteristics and HFE genotype distribution in 133 cirrhotic patients with HCC and 100 cirrhotic patients without HCC.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HCC (n=133)</th>
<th>No HCC (n=100)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 (11)</td>
<td>58 (13)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>113/20</td>
<td>45/55</td>
<td>0.0001</td>
</tr>
<tr>
<td>Aetiology of cirrhosis (%)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Alcohol</td>
<td>76 (57)</td>
<td>57 (57)</td>
<td>NS</td>
</tr>
<tr>
<td>HCV</td>
<td>30 (22)</td>
<td>26 (26)</td>
<td>NS</td>
</tr>
<tr>
<td>HBV</td>
<td>11 (7)</td>
<td>4 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>Mixed†</td>
<td>17 (13)</td>
<td>9 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>Other‡</td>
<td>1 (1)</td>
<td>4 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>HFE genotype (%)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C282Y/C282Y</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>NS</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>0 (0)</td>
<td>2 (2)</td>
<td>NS</td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>NS</td>
</tr>
<tr>
<td>C282Y/WT‡</td>
<td>7 (5)</td>
<td>4 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>H63D/WT‡</td>
<td>41 (31)</td>
<td>38 (38)</td>
<td>NS</td>
</tr>
<tr>
<td>WT/WT†</td>
<td>85 (64)</td>
<td>54 (54)</td>
<td>NS</td>
</tr>
</tbody>
</table>

HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus.

*Alcohol and HCV/alcohol and HBV.
†Primary biliary cirrhosis (n=3), cryptogenic cirrhosis (n=2).
‡WT, wild type.

**Table 2** Distribution of HFE genotypes according to the aetiology of cirrhosis and the presence of HCC in 233 cirrhotic patients (133 with HCC, 100 without HCC).

<table>
<thead>
<tr>
<th>Aetiology of cirrhosis</th>
<th>HCC− (n=133)</th>
<th>HCC+ (n=100)</th>
<th>HCC− (n=97)</th>
<th>HCC+ (n=39)</th>
<th>HCC− (n=13)</th>
<th>HCC+ (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (n=133)</td>
<td>C282Y/C282Y</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C282Y/H63D</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>H63D/H63D</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C282Y/WT‡</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>H63D/WT‡</td>
<td>23</td>
<td>24</td>
<td>11</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>WT/WT†</td>
<td>32</td>
<td>47</td>
<td>16</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>p Value</td>
<td>0.10</td>
<td>0.52</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3** Liver iron loading in 162 cirrhotic patients with and without HFE gene mutations.

<table>
<thead>
<tr>
<th>HFE genotype</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282Y and/or H63D*</td>
<td>37</td>
<td>64</td>
<td>14</td>
<td>11</td>
<td>0.13</td>
</tr>
</tbody>
</table>

*C282Y heterozygotes, H63D heterozygotes, and compound heterozygotes were considered together.
†No mutation=wild-type/wild-type.
with HCC compared with those without HCC.\textsuperscript{4–6}\textsuperscript{11} Willis and colleagues\textsuperscript{21} reported a 7% prevalence of the C282Y homozygous mutation in patients with HCC, significantly higher than that of 0.7% expected in the normal population. It must be stressed however that this study was performed retrospectively on a limited number of patients (n=28) by extracting DNA from archived tissue samples of a population of 181 patients with alcoholic cirrhosis, Lauret and colleagues\textsuperscript{22} found that the prevalence of the C282Y heterozygous mutation was significantly higher in 43 patients with HCC than in 136 without HCC (20.9% and 4.4%, respectively; p=0.002). This result was not observed in another group of 98 patients with viral cirrhosis of whom 34 had HCC. In the present study, the prevalence of the C282Y heterozygous mutation in patients with HCC was lower (5%) and did not differ between patients with alcoholic and viral cirrhosis (6.6% and 2.6%, respectively). Aldersley and colleagues\textsuperscript{23} found a 6.3% prevalence of the C282Y homozygous mutation in 32 patients with HCC compared with 0% in a group of 82 chronic cholestatic liver disease controls, and a 25% prevalence of the C282Y homozygous patients was mild to moderate and strikingly lower than in C282Y homozygous patients. The French population showed that hepatic iron overload in non-HFE disease controls, and a 25% prevalence of the C282Y homozygous mutation in patients with cirrhosis. These mutations did not appear to be associated with an increased risk of HCC in patients with cirrhosis without iron overload.

However, liver iron loading did not differ between cirrhotic patients with and without HCC in the present study, a finding in keeping with our previous work where we did not find a significant relation between hepatic iron content and the occurrence of HCC in patients with alcoholic or hepatitis C virus related cirrhosis.\textsuperscript{24} Thus the role of liver iron overload in the development of HCC must be elucidated.

In conclusion, the similar prevalence of C282Y and H63D mutations in cirrhotic patients with or without HCC suggests a lack of association between HFE gene mutations and HCC in patients with cirrhosis. These mutations do not appear to contribute to, but do not fully explain, hepatic iron accumulation as observed with its disease other than GH. Indeed, heterozygosity for HFE gene mutations seems to contribute to, but does not fully explain, hepatic iron accumulation in patients with chronic hepatitis C.\textsuperscript{25}\textsuperscript{26}\textsuperscript{27}\textsuperscript{28}\textsuperscript{29} Also, it does not seem to influence liver iron content or fibrosis in alcoholic patients.\textsuperscript{26} Conversely, we cannot exclude the fact that absence of correlation between liver iron loading and HFE genotype may be due to lack of reliability of hepatic iron content determinations in cirrhotic patients. Indeed, a considerable intrahepatic variability in iron concentration and frequent overlap in these values between GH and end stage cirrhosis have been reported.\textsuperscript{27}\textsuperscript{28} Liver iron overload has been reported in non-tumoral liver of most patients with HCC developed in non-cirrhotic liver, suggesting that it may act as a co-carcinogenic agent.\textsuperscript{11} 

References


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