The prevalence of HFE C282Y gene mutation is increased in Spanish patients with porphyria cutanea tarda without hepatitis C virus infection

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C282Y, H63D, HCV, HFE, iron overload, porphyria cutanea tarda

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Abstract

Objectives To investigate the role of C282Y and H63D mutations, and hepatitis C virus (HCV) infection in the pathogenesis of porphyria cutanea tarda (PCT).

Design Prospective case-control study.

Setting A large clinical and research institute for the study and treatment of cutaneous diseases in Barcelona, Spain.

Patients Ninety-nine consecutive patients with PCT and one hundred and twenty-six control patients (76 healthy subjects and 50 patients chronically infected with HCV), were recruited.

Main outcome measures The frequency of the C282Y and H63D mutations in patients with PCT vs. controls and the relationship of these mutations with HCV infection, and iron status, as judged by serum iron, liver iron and ferritin levels.

Results C282Y mutation was significantly increased in PCT patients. This mutation was more frequent among non-HCV-infected patients. Increased ferritin levels and hepatic iron overload were also observed in PCT patients with heterozygous C282Y state. H63D mutation was only significantly increased among PCT patients with chronic hepatitis C infection. No significant iron overload was observed in patients with H63D mutation.

Conclusions This study confirms the high frequency of C282Y mutation in patients with PCT and its relationship with iron overload. The C282Y mutation has a relevant role in Spanish patients with PCT not associated with HCV chronic infection. On the other hand, the prevalence of the H63D mutation seems not to be increased in patients with PCT. The possibility of an association between HCV infection and H63D mutation in inducing PCT can be hypothesized.

Introduction

Porphyria cutanea tarda (PCT), a disease presenting with vesiculo-bullous eruption on photo-exposed areas of the skin, is caused by a congenital or acquired reduction in the activity of the enzyme uroporphyrinogen decarboxilase (URO-D). In familial PCT, a marked reduction of the enzyme activity is detected, caused by mutations of the URO-D gene. This form of porphyria is inherited as an autosomal dominant trait and affects approximately between 20 and 25% of patients. In sporadic PCT, the most common form of porphyria, a reduction of URO-D is demonstrated exclusively in hepatocytes, through inactivation of the normal enzyme. PCT is associated with liver-cell damage. Various factors such as alcohol, oestrogens and hepatitis C virus infection may precipitate the disease.

In most patients with sporadic PCT, liver iron deposits (hepatic siderosis) are present. The clinical manifestations
of the disease may be precipitated by liver iron overload, and phlebotomy (an indirect treatment that leads to iron withdrawal) is the treatment of choice for PCT patients.7–10 The cause of hepatic iron overload in sporadic PCT is unknown.

The possibility that genetically predisposed individuals have more susceptibility to sporadic PCT has been hypothesized.11 The observation of a possible association with HLA locus A antigens and PCT led to a systematic search for a susceptibility gene telomeric to the HLA complex. The HFE gene (previously named HLA-H) that encodes an HLA class I-like molecule as a gene candidate for haemochromatosis was identified by Feder et al.12 in 1996. Several mutations of the HFE gene have been identified: in approximately 85% of chromosomes (83% of patients) from patients with haemochromatosis, a point mutation in the HFE gene that replaces cysteine amino acid at position 282 by a tyrosine (C282Y) is detected. A second mutation, which replaces histidine 63 by aspartic acid (H63D), over-represented in haemochromatosis, was also identified. However, this latter mutation seems to be fairly common in the general population and, by itself, is not associated with iron overload.

The identification of mutations on the HFE gene in haemochromatosis has allowed the study of the role of these mutations in PCT patients. An increased frequency of the C282Y mutation in PCT patients was initially noted13 and additional studies confirmed these results.14–17 More recently, the association between the H63D mutation and PCT has also been described.18,19

The present study aimed to determine the prevalence of HFE mutations in PCT patients in Spain, to analyse the role of these mutations in the degree of iron overload and to assess the relationship between HFE mutations and HCV infection as triggering factors of sporadic PCT.

**Patients and methods**

**Patients**

The study was carried out on 99 consecutive Spanish patients with sporadic PCT (89 male patients; mean age of 58, ranging from 31 to 81 years, and 10 female patients; mean age of 59, ranging from 37 to 77). The diagnosis of PCT was based on characteristic clinical and laboratory features. All patients had skin lesions suggestive of PCT such as blistering or skin fragility on the dorsum of the hands. A marked increase of uro- and heptacarboxy-porphyrins in urine and presence of isocoproporphyrin in faeces was also found in all patients. None of the patients had a clinical picture or a family history of haemochromatosis or PCT. No history of environmental exposure to hepatotoxic or porphorygenic chemicals or toxins was recorded. Five women had a history of oestrogen therapy.

Serum ferritin levels were determined before treatment in all patients. For measurement of hepatic iron concentration, a needle liver biopsy was carried out in 41 patients. In all patients, serological markers for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection were determined. Alcohol abuse was defined by present or past alcohol intake higher than 80 g/day for more than 5 years.

A control group of 126 subjects was selected. Seventy-six healthy subjects (group A) and 50 patients chronically infected with HCV (group B) were randomly selected from the pool of subjects available in the laboratory. None of the controls had a history of PCT or haemochromatosis.

All case and control subjects gave their informed and written consent according to a protocol reviewed and approved by the local Ethics Committee (Helsinki Convention).

**Methods**

Mutation analysis of the HFE gene was performed on DNA extracted from frozen stored samples of whole blood using the QIAamp Blood Kit (Qiagen, Santa Clarita, CA, USA) and amplified by PCR, which was followed by restriction endonuclease digestion. For both the C282Y and the H63D mutations, known homozygous normal, heterozygous, and homozygous abnormal controls were run simultaneously with each batch of PCT patients. The primers used for the touch down PCR were as follows: to amplify exon 4, 5′-TGCCCTCTTGTGGATTGACAC-3′ and 5′-CAGATCCCTCATCTCACTGCCC-3′ for exon 2, 5′-TGATGCTGTCCTGCTCCAGGTT-3′ and 5′-ACCCCTTGCTGTGGTGAT-3′. The restriction enzymes used were RsaI for exon 4 and MboI for exon 2, and the resulting fragments were separated by electrophoresis in an acrylamide gel (PAGE) and visualized by ethidium bromide staining.

The conditions for running the PCR were 10′ 96°C (20′′ 96°C5′ 65°C-3′ 72°C) × 5 (20′′ 96°C5′ 60°C-3′ 72°C) × 20 (20′′ 96°C1′ 55°C-3′ 72°C) × 9, 10′ 72°C.

Standard biochemical tests and porphyrin analysis were performed in the hospital laboratories. Quantitative assessment of urinary total porphyrin was carried out by spectrofluorimetry;20 urinary and faecal porphyrin profiles were analysed by high pressure liquid chromatography (HPLC).21 Hepatitis B virus infection was investigated by hepatitis B surface antigen and by the antibody to hepatitis B core antigen (Abbott Laboratories, North Chicago, IL, USA). Detection of HCV RNA was performed by PCR as previously described.22

Liver iron concentration was measured by atomic absorption spectrophotometry.23 The results were expressed
were measured by standard methods. Serum iron (μg/dL; normal range 50–150) and ferritin (ng/L; normal range 20–300 in male subjects and 15–200 in female subjects) were measured by standard methods.

**Statistical analysis**

The significance of the differences between frequencies for patients and controls was determined by the chi-square test (with Yates’ correction). When iron levels were analysed the non-parametric Mann–Whitney U-test was used to compare median values.

**Results**

**Mutational analysis of HFE**

The frequency of HFE mutations and genotypes of patients and controls is shown in Table 1. The C282Y mutation was present in 8% of chromosomes in patients with porphyria cutanea and in 3.2% of controls ($P < 0.02$). When the distribution of the mutation was considered in individuals, the frequency in PCT patients (16.2%) was significantly increased in comparison with the control group (6.3%; $P < 0.03$). None of the subjects included in the study was homozygous for the C282Y mutation.

The H63D mutation was found in 31.8% of chromosomes from patients with PCT, which was not significantly increased over controls (22.6%; $P = 0.119$). This missense mutation was present in heterozygosity in 43.4% of PCT patients in comparison with the control group (37.3%; $P = 0.067$). Homozygosity was present in 10.1% of PCT patients compared with 4% in controls ($P = 0.067$). When all patients with the H63D mutation, either in its heterozygous or homozygous form, were compared to controls (53.5 vs. 41.3%), no significant difference was observed ($P = 0.089$).

The presence of at least one of the HFE mutations was observed more frequently in PCT patients than in controls (62.6 vs. 45.2%; $P < 0.01$). Seven patients with PCT (7%) and three subjects among controls (2.4%) were compound heterozygotes for C282Y and H63D mutations ($P = 0.17$).

No correlation between HFE mutations and alcohol intake was found (data not shown).

**HFE mutations in PCT patients according to the hepatitis C virus infection status**

Seventy-five patients (75%) with PCT were HCV-positive. None was actively infected by hepatitis B virus (HBV) but six had markers of infection in serum (positive anti-HBc antibodies).

The heterozygous C282Y change was twice as frequent in HCV-negative than in HCV-positive PCT patients (25 and 13.3%, respectively) but without reaching statistical significance ($P = 0.177$) (Table 2). However, when the frequency of this mutation in non-infected PCT patients was compared with the control group A (healthy

<table>
<thead>
<tr>
<th>Alleles</th>
<th>PCT (n = 99)</th>
<th>Controls (n = 126)</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282Y</td>
<td>6/198 (8%)</td>
<td>7/252 (2.8%)</td>
<td>0.02</td>
</tr>
<tr>
<td>H63D</td>
<td>63/198 (31.8%)</td>
<td>57/252 (22.6%)</td>
<td>0.119</td>
</tr>
</tbody>
</table>

**Table 1** Frequency of HFE genotypes in PCT patients and controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>PCT (n = 24)</th>
<th>Controls (n = 75)</th>
<th>Pct</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282/C282</td>
<td>18/24 (75%)</td>
<td>65/75 (87%)</td>
<td></td>
</tr>
<tr>
<td>C282/C282Y</td>
<td>6/24 (25%)†</td>
<td>10/75 (13%)††</td>
<td></td>
</tr>
<tr>
<td>H63/H63</td>
<td>15/24 (62.5%)</td>
<td>31/75 (41%)</td>
<td></td>
</tr>
<tr>
<td>H63/H63D</td>
<td>7/24 (29%)‡‡</td>
<td>36/75 (48%)</td>
<td></td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>2/24 (8%)‡‡ ,§§</td>
<td>8/75 (11%)¶¶</td>
<td></td>
</tr>
<tr>
<td>H63D/H63</td>
<td>15/24 (62.5%)</td>
<td>31/75 (41%)</td>
<td></td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>2/24 (8%)‡‡ ,§§</td>
<td>8/75 (11%)¶¶</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Frequency of HFE genotypes in PCT patients and controls with regard to HCV infection status

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>PCT (n = 24)</th>
<th>Controls (n = 75)</th>
<th>Pct</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282/C282</td>
<td>18/24 (75%)</td>
<td>65/75 (87%)</td>
<td></td>
</tr>
<tr>
<td>C282/C282Y</td>
<td>6/24 (25%)†</td>
<td>10/75 (13%)††</td>
<td></td>
</tr>
<tr>
<td>H63/H63</td>
<td>15/24 (62.5%)</td>
<td>31/75 (41%)</td>
<td></td>
</tr>
<tr>
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<td>7/24 (29%)‡‡</td>
<td>36/75 (48%)</td>
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</tr>
<tr>
<td>H63D/H63D</td>
<td>2/24 (8%)‡‡ ,§§</td>
<td>8/75 (11%)¶¶</td>
<td></td>
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</tr>
<tr>
<td>H63D/H63D</td>
<td>2/24 (8%)‡‡ ,§§</td>
<td>8/75 (11%)¶¶</td>
<td></td>
</tr>
</tbody>
</table>

Group A: controls without HCV; group B: controls with chronic infection of HCV.

*P = 0.012; compares group PCT/HCV– with group A; †P = 0.177; compares group PCT/HCV+ with group PCT/HCV+.

†P = 0.188; compares group PCT/HCV+ with group B; §P = 0.896; compares both control groups (A and B).

¶P = 0.371; compares group PCT/HCV+ with group A; **P = 0.166; compares group PCT/HCV– with group PCT/HCV+.

††P = 0.068; compares group PCT/HCV+ with group B; †††P = 0.52; compares group PCT/HCV+ with group A.

]**P = 0.74; compares group PCT/HCV– with group PCT/HCV+; †††P = 0.56; compares group PCT/HCV+ with group B.

***P = 0.69; compares group PCT/HCV– with group A; ††††P = 0.115; compares group PCT/HCV– with group PCT/HCV+.

‡‡‡P = 0.02; compares group PCT/HCV+ with group B.
subjects), a statistical difference was observed (25 vs. 6%; \( P = 0.012 \)). In contrast, in HCV positive patients the frequency of C282Y mutation did not differ from the HCV-positive control group B (13 vs. 6%; \( P = 0.188 \)).

The heterozygous H63D mutation was more frequent in HCV positive patients (48%) than in both HCV-negative patients (29%; \( P = 0.166 \)) and the HCV-positive control group (30%; \( P = 0.068 \)) without reaching statistical significance. Similarly, no differences were observed in the frequency of homozygous H63D mutation regarding HCV infection.

On the one hand, when patients with heterozygous and homozygous H63D mutation were gathered in the same group, this mutation was more prevalent among HCV positive PCT patients when compared to HCV positive controls (58 vs. 36%; \( P = 0.02 \)) (Table 2). On the other, as observed when HCV was not considered (Table 1), no statistical difference was observed when HCV-negative PCT patients were compared to HCV-negative controls (37 vs. 45%; \( P = 0.69 \)).

**Correlation of the iron phenotype with the HFE genotype**

The hepatic iron concentration and ferritin levels were highest in PCT patients who were heterozygous for the C282Y mutation (Table 3). Serum iron levels were increased in all PCT patients but no statistical significance could be established according to the HFE genotype. Similarly, no statistically significant increase in serum iron, ferritin and hepatic iron was observed when the H63D mutation was present.

### Table 3 Iron phenotype in PCT patients

<table>
<thead>
<tr>
<th></th>
<th>C282/C282</th>
<th>C282/C282Y</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver iron (µg/100 mg)</td>
<td>84.7 (34)</td>
<td>242 (7)</td>
<td>0.019</td>
</tr>
<tr>
<td>Serum iron (µg/dL)</td>
<td>128 (78)</td>
<td>150 (12)</td>
<td>0.072</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>353 (61)</td>
<td>648 (10)</td>
<td>0.032</td>
</tr>
<tr>
<td><strong>H63/H63</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver iron (µg/100 mg)</td>
<td>116.2 (19)</td>
<td>106.3 (17)</td>
<td>0.346</td>
</tr>
<tr>
<td>Serum iron (µg/dL)</td>
<td>108 (42)</td>
<td>134 (40)</td>
<td>0.697</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>367 (32)</td>
<td>356 (33)</td>
<td>0.798</td>
</tr>
<tr>
<td><strong>H63/H63</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver iron (µg/100 mg)</td>
<td>116.2 (19)</td>
<td>69.4 (5)</td>
<td>0.406</td>
</tr>
<tr>
<td>Serum iron (µg/dL)</td>
<td>108 (42)</td>
<td>135 (8)</td>
<td>0.233</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>367 (32)</td>
<td>625 (6)</td>
<td>0.136</td>
</tr>
</tbody>
</table>

Values are the medians. Normal liver iron levels are < 50 µg/100 mg dry weight. Normal serum iron values are 50–150 µg/dL and normal ferritin values are 20–300 ng/L. Values in brackets represent the number of patients.

**Discussion**

The identification of the HFE gene and the association of some mutated forms with iron overload in haemochromatosis have prompted the study of the presence of these mutations in other disorders in which there is an increase of iron deposits. Several studies suggested that HFE mutations described in haemochromatosis could also be related to iron overload observed in sporadic PCT, and in some way could contribute to triggering of the clinical onset of the disease.

Overall, mutant HFE alleles were detected in 63% of the PCT patients we genotyped (Table 1), being in the same order than previously reported and significantly higher than that found in the control group (45%).

Our study shows that the C282Y mutation prevalence is significantly increased in sporadic PCT Spanish patients. However, H63D mutation prevalence was not significantly increased in these patients. The heterozygous C282Y mutation has been found to be the most relevant HFE mutation in PCT patients from North Europe and America, but reports from the Mediterranean area argue that H63D is the strongest associated mutation. Similarly, homozygosity for the C282Y mutation has been associated with an earlier onset of skin lesions in both familial and sporadic PCT.

The C282Y mutation is the most common defect associated with hereditary haemochromatosis in Spain. This result also differs from those reported by Italian investigators, who found a lower frequency of C282Y among haemochromatosis patients. The differences found between Spanish and Italian studies among PCT and haemochromatosis patients may be due to the Spanish Celtic genetic background.

Hepatitis C virus infection is a prevalent cause of liver disease in PCT patients and an important triggering factor of this cutaneous disease in predisposed individuals in the Mediterranean area. As HCV infection is frequent in Spanish PCT patients, we examined a control group of patients with HCV chronic active hepatitis, to rule out a direct association between the C282Y mutation and HCV infection. The prevalence of HFE mutations in the control group with HCV hepatitis was almost identical to that observed in controls without the infection, suggesting that the increased prevalence of C282Y is directly associated with PCT. The prevalence of C282Y was higher when PCT patients were not infected by HCV. Therefore, the C282Y mutation might play a relevant role as a triggering factor of PCT, particularly when no HCV infection is detected.

Conversely, when PCT patients are chronically infected by HCV, prevalence of the H63D mutation is increased. To our knowledge, this association has not been previously described, although a similar trend without reaching
protein behaves similarly to the wild-type protein. In villous cells to absorb an excess of iron. On the other hand, poor in iron, will induce cells differentiating into mature urocytes to an abnormal iron absorption. Duodenal crypt cells, synergize to flare up clinical PCT.

Conversely, the prevalence of the H63D mutation is only present. Similar results have been observed by other authors. However, in our series, the presence of H63D did not seem to correlate with the iron status of PCT patients, as judged by serum iron, liver iron and ferritin levels. HFE associates with β₂-microglobulin and facilitates the uptake of transferrin bound iron by duodenal crypt cells. HFE mutations that impair this function contribute to an abnormal iron absorption. Duodenal crypt cells, poor in iron, will induce cells differentiating into mature villous cells to absorb an excess of iron. On the other hand, several authors have found that the H63D mutant protein behaves similarly to the wild-type protein. In the same way, the role of H63D in haemochromatosis has not been clearly shown in several clinical studies. Therefore, several lines of evidence indicate that the H63D mutation could cause a subtler abnormality of iron metabolism than C282Y. H63D mutation may interact with other host molecules such as the transferrin receptor or may also interfere with HCV metabolism inducing a reversible urogen decarboxylase inactivation.

HFE mutations, HCV infection, oestrogen treatment and/or alcohol intake was found in the majority of our patients. However, in a remaining 6% of them no clear exposure to triggering factors could be found. Similarly, HFE mutations and other triggering factors of PCT may be found in many individuals without overt PCT, thus suggesting that other genetic or environmental factors may be involved.

In conclusion, this study shows that the prevalence of the C282Y mutation is increased in sporadic PCT Spanish patients. We found this increase to be more pronounced in non-HCV infected PCT patients. A correlation of this mutation with hepatic iron overload was also observed. Conversely, the prevalence of the H63D mutation is only increased in HCV-infected PCT patients. This finding suggests that the H63D mutation needs to be associated with other factors to trigger PCT, possibly because of its inability to increase iron status by itself.

References
linked to Porphyria Cutanea tarda in Mediterraneans is His63Asp. Hepatology 1999; 30: 819–820.


