Hemochromatosis (HFE) Gene Mutations and Response to Chloroquine in Porphyria Cutanea Tarda

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Objective: To examine the role of hemochromatosis (HFE) gene mutations, which are associated with porphyria cutanea tarda (PCT), in the therapeutic response to chloroquine.

Design: We retrospectively analyzed a database (Excel version 2001 [Microsoft Excel, Redmond, Wash]; date range of search, 1985-1999) of chloroquine-treated patients with PCT on whether HFE mutations (C282Y and H63D) might have influenced the clinical response, urinary porphyrin excretion, liver enzyme activities, and serum iron markers. Serum samples and corresponding complete sets of data before and after therapy were available in 62 of 207 patients with PCT who were treated exclusively with chloroquine.

Results: Of the 62 German patients with PCT, 37 (60%) carries HFE mutations. Chloroquine therapy was accompanied by clinical remission and reduced urinary porphyrin excretion (P<.001) in the 24 patients (39%) with HFE wild type as well as in 35 HFE heterozygous patients with PCT (56%). Decreases of serum iron markers following chloroquine therapy were limited to patients with PCT and HFE wild type. All patients homozygous for the C282Y mutation (3 [5%] of 62) had high serum iron, ferritin, and transferrin saturation and failed to respond to chloroquine treatment.

Conclusions: The therapeutic response to chloroquine was not compromised by C282Y heterozygosity and compound heterozygosity of HFE mutations. Because HFE C282Y homozygotes (+/+ ) did not respond to chloroquine and a decrease in serum iron concentration was limited to patients with PCT and HFE wild type, phlebotomy should be first-line therapy in patients with PCT and HFE mutations.

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ORPHYRIA CUTANEA tarda (PCT) is associated with impaired function of the enzyme uroporphyrinogen de-carboxylase (URO-D) in the liver, leading to characteristic alterations of urinary heme precursors and to typical lesions of sun-exposed skin.1-4 Iron overload is common in PCT and much evidence exists that iron is an inhibitory cofactor of URO-D activity in hepatocytes.5,6 Accordingly, based on clinical studies, iron removal is an efficient treatment for patients with PCT, resulting in improvement of hepatic URO-D activities.7 Alcohol, hormones, drugs, human immunodeficiency virus (HIV), and hepatitis C virus infection are well-known trigger factors responsible for the precipitation of PCT and the associated hepatic alterations.

In a study from northern Italy, however, C282Y mutations occurred as frequently in patients with PCT as in controls (1.5%), whereas the H63D mutation was significantly increased in patients with PCT.10 In Japanese and Bulgarian patients, no C282Y mutations were found.20,21 Thus regional and national variations in prevalence of the C282Y mutation appear to contribute to the geographically different risk for the manifestation of PCT via iron accumulation.

To our knowledge, there is no information on clinical implications of HFE mu-
Urinary porphyrins were separated by thin-layer chromatog-
sets of data before and after therapy to analyze the therapeutic
to perform
and 36 men; mean±SD [median, range] age, 51±13 [49, 21-
the present study, 62 consecutive patients with PCT (26 women
and 1999. They were monitored in a follow-up program per-
quine diphosphate (125-250 mg twice weekly) between 1985
Dresden, Germany, who were treated exclusively with chloro-
tions in chloroquine-treated patients with PCT. Therefore,
we performed a longitudinal study of 62 HFE-
genotyped patients with PCT before and after treatment
with low-dose chloroquine diphosphate, 125 to 250 mg
twice weekly, which is an established therapy for the dis-
order.22-24

<table>
<thead>
<tr>
<th>Table 1. Allele and Genotype Frequencies of HFE Mutations in Patients With PCT and Healthy Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele</td>
</tr>
<tr>
<td>C282Y</td>
</tr>
<tr>
<td>H63D</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>C282Y+/+</td>
</tr>
<tr>
<td>C282Y+/−</td>
</tr>
<tr>
<td>C282Y/H63D</td>
</tr>
<tr>
<td>H63D+/+</td>
</tr>
<tr>
<td>H63D+/−</td>
</tr>
<tr>
<td>WT+/+</td>
</tr>
</tbody>
</table>

Abbreviations: PCT, porphyria cutanea tarda; WT, wild type.
*Data are number (percentage) of subjects positive for the mutation unless otherwise specified.
†Living in Germany for at least 2 generations.

Heterozygosity for the C282Y (C282Y+/−) and H63D
(H63D+/−) mutation or compound heterozygosity (H63D/
C282Y) was significantly more frequent in patients with
PCT compared with healthy controls (9 [15%] of 62 vs
3 [3%] of 115 \([P=.007]\); 18 [29%] of 62 vs 12 [10%] of
115 \([P=.003]\); and 8 [13%] of 62 vs 0 of 115 \([P=.001]\),
respectively) (Table 1). Whereas homozygosity for the
C282Y mutation (C282Y+/+) was detected in 3 patients
with PCT (3 [5%] of 62 vs 0 of 115; \(P=.08\)), homozygosity
for the H63D mutation was found in neither patients nor
controls (Table 1). When analyzing data on HFE geno-
type and serum iron markers, strikingly high values for
serum iron, ferritin, and transferrin saturation were found
in all 3 patients homozygous for the C282Y mutation
before and after chloroquine therapy (\(P<.05\)) (Table 2).
Interestingly, one of these patients did not respond to chlo-
roquine, as characterized by persisting high urinary porphyrins
and skin lesions, and the other 2 homozygous patients
initially improved clinically and biochemically but relapsed
within 1 year and were therefore considered as nonresponders.
Remission and decrease of serum iron markers were achieved in all 3 patients after
switching to phlebotomy.

Patients with PCT and wild-type HFE and those
heterozygous or compound heterozygous for the C282Y
or H63D mutation responded to chloroquine therapy by
sustained complete remission of the skin lesions, de-
crease of liver enzyme activities (ALT and AST), and
reduced excretion of urinary porphyrins. To further ana-
lyze the role of heterozygosity of HFE mutations, the 3
homozygotes (C282Y/C282Y) were excluded and the
remaining 35 heterozygotes were com-
pared with the 24 patients with PCT but with wild-type
HFE (Table 2 and Table 3). In the pretreatment pe-
riod, patients with PCT and HFE heterozygosity
(282Y/WT, C282Y/H63D, or H63D/WT) did not have
higher levels of serum iron markers than those with

METHODS

The study population comprised 207 patients with PCT who
lived in an area of approximately 260 km² in and around
Dresden, Germany, who were treated exclusively with chloro-
quine diphosphate (125-250 mg twice weekly) between 1985
and 1999. They were monitored in a follow-up program per-
formed in a specialized outpatient clinic of the Department of
Dermatology, Hospital Dresden-Friedrichstadt, Dresden.25
For the present study, 62 consecutive patients with PCT (26 women
and 36 men; mean±SD [median, range] age, 51±13 [49, 21-
80] years) were included based on (1) available serum samples to
perform HFE genotyping and (2) corresponding complete sets of data before and after therapy to analyze the therapeutic
response to chloroquine.

METHODS

Urinary porphyrins were separated by thin-layer chromatog-
raphy and measured by spectrophotometric absorption.26
Using a questionnaire, daily alcohol consumption and intake of
hormones were recorded in all patients. At the time of serum
sampling, prior to treatment, PCT was overt in all patients. Overt
disease included typical skin lesions accompanied by charac-
teristic patterns of urinary heme precursors. Response to therapy
was defined clinically by remission of skin lesions and re-
flected biochemically by decreased excretion of uroporphyrin
and heptacarboxyphorphyrin. Liver enzyme activities (alamin-
aminotransferase [ALT] and aspartate aminotransferase [AST])
and the concentrations of serum iron, transferrin, and ferritin
were measured in all subjects before and after therapy.27

Hepatitis virus markers were analyzed as described pre-
viously.28 Because our subjects lacked clinical evidence for im-
munodeficiency and because human immunodeficiency virus
(HIV) infection is relatively rare in Saxony (former East Ger-
many), we did not search for HIV infection (eg, by testing for
anti-HIV antibodies) in these patients with PCT.

RESULTS

Patients were treated with chloroquine diphosphate (Chlo-
rocin, Berlin-Chemie, Berlin, Germany), 125 to 250 mg twice
weekly, with a median treatment time of 16 months (range,
12-26 months). All patients were advised to avoid alcohol. Other
dietary restrictions were not recommended. During the pe-
riod of chloroquine medication, neither phlebotomy nor hep-
titis virus treatment was performed.

Genomic DNA was extracted from serum samples and used
as template for a polymerase chain reaction–based assay test-
ing for the C282Y and H63D mutations as described previ-
ously.27 The frequencies of the C282Y and H63D mutations in
patients with PCT were compared with a control group of 115
healthy volunteers (71 men and 44 women; mean±SD [me-
dian, range] age, 58±13 [58, 22-89] years) without any known
liver or skin disease who were recruited from a general dental
practice of the same geographical area.

The significance of the differences between the frequen-
cies of C282Y and H63D mutations for patients with PCT and
controls was determined by \(x^2\) analysis. Analysis of variance
was applied to data on HFE genotype and serum iron and trans-
ferrin saturation. Statistical significance of differences was de-
termined by the \(t\) test, and the \(x^2\) test was used to analyze as-
sociations among categorical variables. The significance level
was defined as \(P<.05\).
wild type alone (Table 2). Baseline characteristics, urinary porphyrin excretion, liver enzymes (ALT and AST), other risk factors (alcohol and hormone intake and rates of hepatitis C virus infection), and the cumulative dose of chloroquine did not differ among these groups (Table 3). In the group of 24 patients with wild-type HFE genes, intraindividual comparison before and after treatment showed a highly significant decrease of urinary porphyrin excretion and liver enzymes (ALT and AST) and a slight but significant decrease of serum iron, ferritin, and transferrin saturation (Table 2). In contrast, among the 34 heterozygous patients with PCT (C282Y/WT, C282Y/H63D, and H63D/WT), serum iron and transferrin saturation were not different before and after therapy, although a comparable decrease of urinary porphyrin excretion and liver enzymes (ALT and AST) was observed (Table 2).

Chloroquine therapy has been shown to be as safe and effective as, but more convenient than, phlebotomy in the treatment of patients with overt PCT.22-24 Phlebotomy is more invasive and time-consuming and can be accompanied by hemodynamic reactions. At present, the decision on whether to use chloroquine or phlebotomy for PCT seems to be more empirical than evidence based. A follow-up and reevaluation of chloroquine-

Table 2. Intraindividual Biochemical Differences Between PCT Patients With HFE Homozygotes (C282Y+/+), Heterozygotes (C282Y+/−, H63D+/−, C282Y/H63D), and Wild Type (WT+/+) Before and After Chloroquine Therapy*

<table>
<thead>
<tr>
<th>HFE Genotype Characteristic</th>
<th>Before Therapy</th>
<th>After Therapy</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary porphyrins, µg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C282Y +/−, H63D +/−, H63D/C282Y</td>
<td>3283 ± 3018</td>
<td>200 ± 69</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>WT +/+</td>
<td>2479 ± 1802</td>
<td>172 ± 70</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C282Y +/+</td>
<td>2632 ± 576</td>
<td>656 ± 346</td>
<td>ND</td>
</tr>
<tr>
<td>Serum ALT, U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C282Y +/−, H63D +/−, H63D/C282Y</td>
<td>73.8 ± 39.6</td>
<td>37.2 ± 39.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>WT +/+</td>
<td>71.4 ± 35.4</td>
<td>31.2 ± 15.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C282Y +/−</td>
<td>59.4 ± 21.0</td>
<td>39.6 ± 23.4</td>
<td>ND</td>
</tr>
<tr>
<td>Serum transferrin saturation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C282Y +/−, H63D +/−, H63D/C282Y</td>
<td>52.8 ± 23.4</td>
<td>29.4 ± 16.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>WT +/+</td>
<td>51.0 ± 21.6</td>
<td>30.0 ± 15.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C282Y +/−</td>
<td>39.0 ± 4.2</td>
<td>39.0 ± 4.2</td>
<td>ND</td>
</tr>
<tr>
<td>Serum iron, µg/d (µmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C282Y +/−, H63D +/−, H63D/C282Y</td>
<td>145 ± 50 (26 ± 9)</td>
<td>134 ± 45 (24 ± 8)</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>WT +/+</td>
<td>145 ± 45 (26 ± 8)</td>
<td>123 ± 22 (22 ± 4)</td>
<td>.01</td>
</tr>
<tr>
<td>C282Y +/−</td>
<td>234 ± 45 (42 ± 8)</td>
<td>212 ± 56 (38 ± 10)</td>
<td>ND</td>
</tr>
<tr>
<td>Serum transferrin saturation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C282Y +/−, H63D +/−, H63D/C282Y</td>
<td>43 ± 15</td>
<td>43 ± 19</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>WT +/+</td>
<td>45 ± 14</td>
<td>38 ± 11</td>
<td>.03</td>
</tr>
<tr>
<td>C282Y +/−</td>
<td>87 ± 13</td>
<td>85 ± 28</td>
<td>ND</td>
</tr>
<tr>
<td>Serum ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C282Y +/−, H63D +/−, H63D/C282Y</td>
<td>880 ± 880</td>
<td>572 ± 352</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>WT +/+</td>
<td>1425 ± 1628</td>
<td>748 ± 1100</td>
<td>.002</td>
</tr>
<tr>
<td>C282Y +/−</td>
<td>924 ± 352</td>
<td>1276 ± 440</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ND, not done; PCT, porphyria cutanea tarda.

*Data are mean ± SD unless otherwise specified. Normal ranges are as follows: urinary porphyrins, 200 µg/d; serum ALT, 11 to 24 U/L (women) and 13 to 30 U/L (men); serum AST, 14 to 28 U/L (women) and 16 to 29 U/L (men); serum iron, 307 to 810 µg/dL (women) and 436 to 844 µg/dL (men); serum transferrin saturation, 20% to 55%; and serum ferritin, <330 ng/mL (age <65 years) and <600 ng/mL (age ≥65 years).

Table 3. Comparison of Baseline Data and Risk Factors Between PCT Patients With HFE Heterozygotes (C282Y+/−, H63D+/−, C282Y/H63D) and HFE Wild Type (WT+/+)

<table>
<thead>
<tr>
<th>Patient Characteristic</th>
<th>PCT Patients With HFE Heterozygosity (n = 24)</th>
<th>PCT Patients With HFE Wild Type (WT+/+) (n = 35)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD, y</td>
<td>48 ± 12</td>
<td>55 ± 14</td>
<td>≥.05</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>18/17</td>
<td>16/8</td>
<td>≥.05</td>
</tr>
<tr>
<td>Alcohol intake &gt;60 g/d, No. (%)</td>
<td>10 (29)</td>
<td>11 (46)</td>
<td>≥.05</td>
</tr>
<tr>
<td>Hormone intake, No. (%)</td>
<td>10 (29)</td>
<td>6 (25)</td>
<td>≥.05</td>
</tr>
<tr>
<td>Anti-HCV positive, No. (%)*</td>
<td>2 (7)</td>
<td>2 (11)</td>
<td>≥.05</td>
</tr>
<tr>
<td>Cumulative dose of chloroquine, mean ± SD, g</td>
<td>23 ± 11</td>
<td>23 ± 9</td>
<td>≥.05</td>
</tr>
</tbody>
</table>

Abbreviations: HCV, hepatitis C virus; PCT, porphyria cutanea tarda.

*Of the 35 PCT patients with HFE heterozygosity, 27 were screened for anti-HCV antibodies, and of the 24 patients with HFE wild type, 19 were screened.
treated patients with PCT according to different HFE genotypes has not been reported. Hemochromatosis genotyping can help to further classify patients with PCT and associated hemochromatosis. Significant clinical consequences arise for C282Y homozygotes and, less frequently, compound heterozygotes, since diagnosis of hemochromatosis per se implicates risks of iron-related multiorgan damage (bronze diabetes), and lifelong observation is required. Elevated serum iron markers point toward an association with hemochromatosis, which is found in 2% to 27% of patients with PCT.29-31 In the present study we found 3 (5%) of 62 patients with PCT and hemochromatosis as defined by high serum iron markers and homozygosity for the C282Y mutation. Our data show that chloroquine therapy did not affect the markedly elevated serum iron markers in these patients, suggesting that these patients should be treated with phlebotomy to normalize the disturbed porphyrin metabolism and accumulation of toxic iron.

Chloroquine therapy was accompanied by clinical remission, improved liver enzyme activities, and markedly reduced urinary porphyrin excretion in the 24 patients (39%) with HFE wild type as well as in 35 HFE heterozygous patients with PCT (56%). Interestingly, treatment with the drug decreased serum iron markers in the former but not in the latter group (Table 2). Other factors such as alcohol consumption, ingestion of estrogens, chronic hepatitis C, and the cumulative dose of chloroquine did not correlate with the observed differences (Table 3). In accordance with our observations in PCT patients with HFE wild type, chloroquine significantly reduced serum iron markers and liver iron accumulation in normal and iron overloaded rats.28 The weak base chloroquine elevates the pH in acidic cellular organelles and impairs the release of iron from the transferrin-transferrin receptor complex. Mutations of the HFE protein appear to modulate the function of the transferrin-transferrin receptor in favor of intracellular iron deposition, a process that is possibly opposed by chloroquine.

In the present study, as well as in reports from the United Kingdom, the United States, and Australia, the proportion of heterozygosity for C282Y and compound heterozygosity (C282Y/H63D) was significantly increased in patients with PCT.12,16 Both of these genotypes, and in particular compound heterozygosity (C282Y/H63D), which were significantly more frequent in our patients with PCT (Table 1), were described to increase the risk for iron accumulation and development of clinical hemochromatosis.20,21 Recently, an interesting model was established in which complete as well as incomplete (heterozygous) disruption of the HFE gene caused significant hepatic uroporphyrin accumulation in mice.22 This supports a direct role of heterozygosity for the C282Y mutation in the pathogenesis of PCT.

Furthermore, in our study the overall frequency of the H63D mutation and the genotype H63D/WT were increased significantly compared with controls (20% vs 5.2% and 29% vs 10%, respectively) (Table 1). Heterozygous carriers (H63D/WT) of the H63D mutation were considered to have only a slightly increased risk for iron accumulation.23 Alternatively, the H63D mutation could be associated with hidden mutations that otherwise contribute to the manifestation of PCT.

Because liver biopsy procedures were not performed or because formerly stored liver biopsy specimens of these patients were not available, we were not able to compare hepatic iron content before and after therapy. Furthermore, we could not check for URO-D deficiency (familial PCT) because of lack of access to methodology in the former East Germany. From other studies it is known that one third to one half of patients with PCT in Germany have the familial variant.32 Previously, it was reported that hereditary URO-D deficiency did not play a role in modulating demographic or clinical features of PCT.19 Therefore, it seems unlikely that URO-D deficiency would have affected our results.

We can clearly derive the following conclusions from our investigation: (1) There is a high prevalence of the C282Y and H63D mutations of HFE in patients with PCT from Saxony. (2) Simple or compound heterozygosity of HFE mutations did not affect the therapeutic response to chloroquine in PCT. (3) Because HFE homozygotes did not respond to chloroquine and decrease of serum iron markers was limited to patients with PCT and HFE wild type, phlebotomy should be first-line therapy in patients with PCT and HFE mutations.

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