Hemochromatosis genes and other factors contributing to the pathogenesis of porphyria cutanea tarda


Inherited and acquired factors have been implicated in the pathogenesis of porphyria cutanea tarda (PCT), a disorder characterized by a photosensitive dermatosis and hepatic siderosis. This study, comprising 108 patients with PCT, was intended to define the role of hemochromatosis gene (HFE) mutations in the expression of PCT and to determine the contribution of acquired factors including alcohol, hepatitis C virus (HCV), and estrogen. The 2 known HFE mutations, cysteine 282 tyrosine (Cys282Tyr) and histidine 63 asparagine (His63Asp), were detected by polymerase chain reaction, and anti-HCV immunoglobulin G was detected serologically. Liver biopsies were graded for iron content, inflammation, and fibrosis. Estimates of alcohol and estrogen use were based on a questionnaire. Of the PCT patients tested, 19% were homozygous for the Cys282Tyr mutation; controls were equal to 0.5%. The compound heterozygous genotype was detected in 7% of the PCT patients; controls were less than 1%. The transferrin saturation, serum ferritin, and liver iron burden of all PCT patients were higher than those of nonporphyrinic controls. The highest values were found in PCT patients homozygous for the Cys282Tyr mutation. Of the patients studied, 59% were HCV positive (compared with 1.8% of the population), and 46% consumed more than 70 g of alcohol daily. Of the female patients, 63% were ingesting estrogens. Hepatic damage was most marked in patients with the Cys282Tyr/Cys282Tyr genotype who had HCV and drank heavily. Homozygosity for the Cys282Tyr mutation and HCV are the greatest risk factors for expression of PCT, and in most patients, more than 1 risk factor was identified. It was common for patients with HCV to consume alcohol. Patients with PCT should be screened for HFE mutations and for HCV. (Blood. 2000;95:1565-1571)

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Introduction

Porphyria cutanea tarda (PCT) is the most common type of porphyria in humans. Estimates of prevalence have ranged from 1 in 5000 to 25 000 people. PCT is characterized clinically by a photosensitive dermatosis associated with skin fragility and blistering. The cutaneous photosensitivity is mediated by uroporphyrin and partially decarboxylated porphyrins. The source of these compounds is the liver, where the activity of uroporphyrinogen decarboxylase (URO-D) is diminished. These compounds accumulate in the liver, circulate in plasma, and are excreted in the urine.

Two variants constitute virtually all cases of PCT in adults. Familial PCT (F-PCT) is transmitted as an autosomal dominant trait and accounts for about one-third of cases. Many URO-D mutations have been identified in patients with F-PCT. The activity of URO-D is half normal in all tissues, although porphyrins accumulate only in the liver. Most carriers of mutant URO-D alleles do not express a clinical phenotype unless additional factors are present. Sporadic PCT (S-PCT) accounts for approximately two-thirds of cases. In S-PCT, the decrease in activity of URO-D is restricted to the liver, and no mutations in the URO-D gene have been identified. The concentration of URO-D protein in the liver is normal even though enzyme activity is reduced, which suggests the presence of an enzyme inhibitor. Because rare pedigrees have been reported, with several members displaying signs and symptoms of S-PCT, a genetic factor other than a URO-D mutation may be involved.

The term PCT was first suggested by Waldenström in 1937 to distinguish this form of porphyria from the rare photomutilating congenital erythropoetic porphyria (Gunther’s Disease). The association of liver disease, particularly alcoholic liver disease, and PCT was recognized by Brunsting et al. by 1951. Iron was implicated in the pathogenesis of PCT based on the nearly uniform finding of hepatic siderosis and the beneficial effects of therapeutic phlebotomy. Edwards et al. first suggested that hemochromatosis gene mutations were responsible for the hepatic siderosis in many patients with PCT. This suggestion was later confirmed by mutational analysis of the hemochromatosis gene. The introduction of medicinal estrogen for postmenopausal replacement therapy and contraception led to an increase in the incidence of PCT in women and the recognition that oral estrogens could induce clinical expression of PCT. The discovery of the hepatitis C virus (HCV) and the development of serologic and molecular diagnostic tests revealed an association between HCV-induced liver disease and PCT, but the prevalence of HCV infection in patients with PCT varies widely from country to country.

We studied 108 patients with PCT at the General Clinical Research Center of the University of Utah. We report here the...
relative roles of hemochromatosis gene (HFE) mutations, HCV, alcohol, and estrogens as risk factors for disease expression.

**Patients and methods**

**Informed consent**

All patients with PCT, controls with hemochromatosis, and normal controls were evaluated at the General Clinical Research Center under a protocol approved by the University of Utah Institutional Review Board. Both groups of controls were members of hemochromatosis pedigrees previously reported. All study participants gave written informed consent. Subjects who agreed to undergo a liver biopsy received a careful explanation of the risks associated with this procedure and provided specific written informed consent.

**Diagnosis**

The diagnosis of PCT was based on the presence of a characteristic bullous dermatosis associated with skin fragility and an increase in the 24-hour urinary excretion of uroporphyrin and 7-carboxyl porphyrin. The diagnosis of F-PCT was assigned to patients with approximately half-normal activity of URO-D measured in erythrocyte lysates by a modification of the method of Straka et al. Control samples from normal subjects were included in each assay. The diagnosis of S-PCT was assigned when values did not differ from controls.

**Urine porphyrin determinations**

Urine porphyrins were quantified spectrophotometrically and by high-performance liquid chromatography (HPLC). Blood tests of iron stores

The serum iron concentration and transferrin saturation were measured as previously described. When possible, samples were obtained after subjects had fasted overnight. Serum ferritin concentrations were measured with radioimmunoassay kits (Ramco Laboratories, Houston, TX).

**Liver biopsies**

Hepatic iron stores were assessed by light microscopy (graded on a scale of 0 to 4) according to the method of Scheuer et al. and by atomic absorption spectrophotometry. The normal value for hepatic cellular stainable iron is grade 0 to 1. Normal values for hepatic iron concentration are less than 25 µmol/g dry weight. Histologic grading and staging of HCV was performed by the method of Batts and Ludwig. Grading is a measure of the severity of the necroinflammatory process, and staging refers to the degree of fibrosis present.

**HCV antibody**

Anti-HVC IgG (immunoglobulin G) was detected serologically using a commercially available enzyme-linked immunoabsorbent assay (ELISA 2.0, Chiron, Emeryville, CA). A positive result is designated HCV+ and a negative result HCV−.

**HFE genotyping**

DNA was available from 87 patients with PCT (64 with S-PCT and 23 with F-PCT) and from 56 clinically normal subjects married to members of well-characterized hemochromatosis pedigrees. The ancestral cysteine 282 tyrosine (Cys282Tyr) HFE mutation and the histidine 63 asparagine (His63Asn) mutation were detected using polymerase chain reaction (PCR) amplification as described by Feder et al.

**Estimation of alcohol intake**

Alcohol consumption was estimated with a questionnaire and graded as follows: grade 0: nondrinkers; grade 1: consumption of less than 20 g of alcohol per day; grade 2: consumption of between 20 g and 70 g of alcohol per day for a minimum of 3 consecutive years; grade 3: consumption of more than 70 g of alcohol per day for a minimum of 3 consecutive years.

**Statistics**

Statistical analyses were performed using the Prophet computer software package. Serum iron, transferrin saturation, and serum ferritin concentration values in PCT patients were compared to age- and sex-matched controls with the Wilcoxon signed rank test. Frequencies of the HFE genotypes were compared with the Fisher exact test. To calculate an odds ratio for the genotype Cys282Tyr/Cys282Tyr as a risk factor for PCT, we assumed the frequency of the homozygous mutant genotype among controls to be 0.005. The Mantel-Haenszel test was used to calculate an odds ratio for alcohol, HCV, and estrogens in PCT patients with the Cys282Tyr/Cys282Tyr genotype. We employed age- and sex-matched controls with hemochromatosis but without PCT who were members of well-characterized hemochromatosis pedigrees. We used 3 controls matched by age (plus or minus 5 years) and sex for each patient with PCT.

**Results**

**Individuals studied**

We evaluated 108 patients with PCT from 1977 to 1998. Of these patients, 31 (29%) had F-PCT and 77 (71%) had S-PCT. The F-PCT patients were members of 30 pedigrees. There were 18 males (mean age, 35 years) and 13 females (mean age, 45 years); 3 F-PCT patients were children: 2 brothers aged 6 and 10 years and an unrelated 12-year-old girl. The 2 clinically affected young brothers also had a clinically affected paternal uncle (not studied by us). This was the only F-PCT pedigree we evaluated with more than 1 clinically affected member. Of the 77 patients with S-PCT, 50 were males (mean age, 43 years), and 27 were females (mean age, 43 years). We found 2 siblings with S-PCT, a 53-year-old woman and her 49-year-old brother. Both also had hemochromatosis. Both alleles of URO-D were sequenced in these siblings, and no mutations were identified (data not shown). One female S-PCT patient was 5 years old.

The mean 24-hour urinary secretion of porphyrins in the 29 patients with F-PCT was 4061 µg (range, 749-9376 µg/24 h). The mean value for 24-hour urine porphyrin secretion in the 77 patients with S-PCT was 4932 µg (range, 730-26 921 µg/24 h). The highest 24-hour urine porphyrin excretion, 26 921 µg, was found in a 65-year-old woman who was receiving chemotherapy for metastatic lung cancer. The urine porphyrin profile in both F-PCT and S-PCT was similar, with uroporphyrin and 7-carboxyl porphyrin predominating. Normal subjects studied in our laboratory secrete less than 200 µg/24 h of urinary porphyrins, and the predominant urinary porphyrin is coproporphyrin.

**The iron phenotype**

Of the 108 patients with PCT, 9 had initiated phlebotomy therapy prior to our evaluation. The iron phenotype of the remaining 99 patients and 99 controls matched by age and sex is shown in Table 1. No significant differences in the iron phenotype were detected when patients with S-PCT were compared to patients with F-PCT. Values for the serum iron, transferrin saturation, and serum ferritin concentration in patients with PCT were significantly higher than in controls (Table 1). Hepatic parenchymal cell stainable iron was graded on 89 liver biopsies (60 obtained from males and 29 from females). Only 1 patient had no histologic evidence of
hepatocellular iron stores (grade 0), yet her porphyria improved with phlebotomy therapy. This patient, a 27-year-old nulliparous woman with active systemic lupus erythematosus, was ingesting birth control pills at the time PCT developed. She did not drink alcohol and had no evidence of HCV. Liver biopsies from the remaining patients were graded for iron as follows: grade 1: 30 patients; grade 2: 37 patients; grade 3: 14 patients; and grade 4: 7 patients. The hepatic iron concentration measured by atomic absorption spectrophotometry was normal in the single subject with grade 0 liver iron and in 10 of the 30 patients with grade 1 liver iron.

Genotyping at the HFE locus

HFE genotypes for 87 patients with PCT are shown in Table 2; 19 patients were Cys282Tyr homozygotes. Only 17 of these patients are shown in the table, as 2 pairs of Cys282Tyr homozygous siblings were evaluated; 1 sibling pair had F-PCT, and 1 pair had S-PCT. Homozygosity for the Cys282Tyr mutation was clearly overrepresented. The controls had the same frequency of heterozygosity for the Cys282Tyr mutation. Compound heterozygotes for the Cys282Tyr/His63Asp mutations also were overrepresented, but the number of subjects with this genotype was small. Of the 87 PCT patients included in Table 2, 174 chromosomes are represented. The Cys282Tyr mutation was carried in 53 (30%) of these 174 chromosomes compared with 7 (6%) out of 112 chromosomes (6%) in 56 nonporphyric Utah controls (P < .0001).

We also compared the frequency of the His63Asp mutation in these groups. For this comparison all chromosomes bearing the Cys282Tyr mutation were removed from the calculation because His63Asp mutations have not been detected on chromosomes with the Cys282Tyr mutation.\(^\text{30}\) Of the remaining 121 chromosomes from PCT patients, 31 (26%) carried the His63Asp mutation compared with 12 (11%) out of 105 chromosomes in the controls (P = .007). The frequency of the Cys282Tyr mutation on chromosomes from patients with S-PCT (45/132 [34%]) did not differ significantly from the frequency on F-PCT chromosomes (13/46 [28%]) than on chromosomes from S-PCT (18/132 [14%]) (P = .02).

Correlation of the iron phenotype with the HFE genotype

The hepatic iron concentration, transferrin saturation, and the serum ferritin concentration were highest in PCT patients who were homozygous for the Cys282Tyr mutation (Table 3). Regardless of the HFE genotype, patients with PCT had a higher transferrin saturation and serum ferritin concentration than the controls. The mean liver iron grade and transferrin saturation associated with compound heterozygosity at the HFE locus (Cys282Tyr/His63Asp) were higher than those in simple heterozygotes (Cys282Tyr/WT), but statistical significance could not be established because of the small number of compound heterozygotes.

Hepatitis C virus

Serologic testing for anti-HCV IgG was performed on 71 patients with PCT. There were 42 (59%) seropositive (HCV+) patients. Males were more likely to be HCV+ (36/45 [80%]) than females (6/26 [23%]). There was no significant difference in the frequency of HCV+ between patients with S-PCT (35/56 [63%]) and those with F-PCT (7/15 [47%]) (P = .3). Of the 42 HCV+ patients, 27 (64%) had used intravenous drugs in the past, and 1 patient had received blood transfusions following an automobile accident 17 years before PCT developed. There was no significant difference in the frequency of HCV+ in PCT patients with any HFE genotype, nor was the iron phenotype influenced by HCV status.

Approximately 1.8% of the population of the United States has antibodies against HCV.\(^\text{31}\) We tested 64 PCT patients for both the anti-HCV antibody and the Cys282Tyr mutation. We screened 14 PCT patients who were homozygous for the Cys282Tyr mutation for anti-HCV IgG, and 6 (43%) were HCV+. We also tested 60 nonporphyric hemochromatosis homozygotes, and only 3 (5%) were HCV+. These data clearly indicate that the prevalence of HCV+ in patients with PCT greatly exceeds the prevalence in the general population. The odds ratio of developing PCT in hemochromatosis homozygotes is 14 when HCV antibody is present versus when it is not.

We were able to grade 84 of the 89 liver biopsies for active inflammation and staging of fibrosis. Biopsy samples were taken from 39 HCV+ patients, 20 HCV− patients, and 25 patients not tested for HCV. Results are shown in Table 4. Bridging fibrosis (stage 3) and cirrhosis (stage 4) were most prevalent in HCV+ patients, particularly in heavily iron-loaded patients with PCT who consumed over 70 g of alcohol daily. The effect of the Cys282Tyr HFE mutation on the degree of fibrosis in HCV+ patients was also analyzed. We genotyped 58 chromosomes in patients with stage 0 to 2 fibrosis, and 26% carried the Cys282Tyr mutation. A higher proportion of Cys282Tyr-bearing chromosomes was detected in

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### Table 1. The iron phenotype of 99 patients with PCT and 99 controls

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron (µmol/L)</td>
<td>26.0 ± 1.8</td>
<td>19.3 ± 0.72</td>
<td>.0030</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>44 ± 3</td>
<td>30 ± 1</td>
<td>.0003</td>
</tr>
<tr>
<td>Serum Ferritin (µg/L)</td>
<td>472 ± 42</td>
<td>88 ± 8</td>
<td>.0001</td>
</tr>
</tbody>
</table>

Of the 99 patients studied, 65 were males, and 34 were females. The 65 male and 34 female controls were matched by sex and age (± 5 years) with PCT patients. Controls represented members of hemochromatosis pedigrees who shared no HLA alloantigen with the hemochromatosis homozygote pedigree proband. \(^\text{19}\) P values (two-tailed) were calculated with the paired two sample Wilcoxon signed rank test. Values are expressed as the mean ± SEM (standard error of the mean). Values in parentheses represent the median value.

### Table 2. Frequency of HFE genotypes in PCT patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PCT n = 87</th>
<th>Control n = 56</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys282Tyr/Cys282Tyr</td>
<td>17 (19%)</td>
<td>0</td>
<td>.0001</td>
</tr>
<tr>
<td>Cys282Tyr/WT</td>
<td>13 (15%)</td>
<td>7 (13%)</td>
<td>.0061</td>
</tr>
<tr>
<td>Cys282Tyr/His63Asp</td>
<td>6 (7%)</td>
<td>0</td>
<td>.0447</td>
</tr>
<tr>
<td>His63Asp/His63Asp</td>
<td>6 (7%)</td>
<td>1 (2%)</td>
<td>.2460</td>
</tr>
<tr>
<td>His63Asp/WT</td>
<td>13 (15%)</td>
<td>10 (18%)</td>
<td>.6480</td>
</tr>
<tr>
<td>WT/WT</td>
<td>32 (37%)</td>
<td>38 (68%)</td>
<td>.0003</td>
</tr>
</tbody>
</table>

Controls were members of the Utah hemochromatosis pedigrees, who shared no HLA haplotypes with the pedigree proband.\(^\text{20}\) P values were calculated with the Fisher exact test. WT indicates wild type.
and Methods. Histologic grading and staging of HCV was performed by the method of Batts and Ludwig. Grading is a measure of the severity of the necroinflammatory process, with grade 0 indicating normal. Staging refers to the degree of fibrosis present, with stage 0 indicating normal.

Patients with grade 3-4 fibrosis (6 of 12 genotyped chromosomes [50%]), but this difference did not reach statistical significance (P = .09).

Alcohol
Average daily alcohol consumption was estimated in all 108 patients as follows: grade 0 (consumed no alcohol); 11 (10%) patients, including 3 children; grade 1 (drank only occasionally): 24 (22%) patients (5 men and 19 women); grade 2 (consumed between 20 and 70 g of alcohol daily): 23 (21%) patients (17 men and 6 women); and grade 3 (consumed more than 70 g of alcohol daily): 40 (46%) patients (42 men and 8 women). We found 46 patients with PCT who did not have the Cys282Tyr mutation at either HFE allele (Table 3); 27 of these patients consumed more than 70 g of alcohol daily (grade 3). Neither the ferritin concentration nor the liver iron content in this group of 27 patients differed significantly when compared to patients who were heterozygous for the Cys282Tyr mutation and who were also heavy drinkers (grade 3).

Estrogens
Of the 38 adult women with PCT, 24 (63%) were using oral estrogen preparations when PCT was first detected. None were using transdermal estrogen delivery systems. Of the 13 premenopausal women, 9 (60%; mean age, 33 years plus or minus 9 years) had been taking oral contraceptives for more than 6 months (range, 0.5-15 years). Of the 25 postmenopausal women, 15 (60%) were using transdermal estrogen delivery systems. Of the 13 premenopausal women, 9 (60%; mean age, 33 years plus or minus 9 years) had been taking oral contraceptives for more than 6 months (range, 0.5-15 years). Of the 25 postmenopausal women, 9 (60%; mean age, 33 years plus or minus 9 years) had been taking oral contraceptives for more than 6 months (range, 0.5-15 years). Of the 25 postmenopausal women, 9 (60%; mean age, 33 years plus or minus 9 years) had been taking oral contraceptives for more than 6 months (range, 0.5-15 years).

Women with S-PCT and 2 with F-PCT. In the postmenopausal group, there were 10 women with S-PCT and 5 with F-PCT. Estrogen ingestion was the only identified factor known to be associated with PCT in 9 women (7 with S-PCT and 2 with F-PCT). Five of these women had prominent hepatic steatosis; 1 had estrogen-induced hepatic adenomas, but the porphyrin content of a biopsy specimen taken from the adenoma did not differ from a sample from a normal portion of the liver (data not shown). We compared serum iron concentration, transferrin saturation, serum ferritin concentration, liver iron grade, and HCV status between women with PCT who were taking estrogens and those who were not. No significant differences were noted (data not shown).

Associations between risk factors for PCT
The Cys282Tyr/Cys282Tyr genotype was clearly a risk factor for the development of PCT (odds ratio of 60; 95% confidence limits [CLs], 18.5 and 195.7). To estimate the role of risk factors, such as HCV, alcohol, and estrogens, in Cys282Tyr homozygotes, we compared Cys282Tyr homozygotes with PCT to age- and sex-matched Cys282Tyr homozygotes without PCT (Table 5). Homozygotes for the Cys282Tyr mutation with grade 3 alcohol consumption had higher ferritin values and liver iron content than alcohol abusers who were not homozygotes.

We attempted to evaluate HCV and alcohol as independent risk factors in Cys282Tyr homozygotes, but these 2 factors proved to be highly associated. All HCV+ homozygotes had grade 2-3 alcohol consumption, and only 2 alcohol users were HCV−. Estrogen use, however, did prove to be a risk factor independent of HCV status. Estrogen use in Cys282Tyr homozygous women without HCV was

Table 3. Influence of HFE genotype on the iron phenotype in PCT patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male/Female</th>
<th>Serum Iron (µmol/L)</th>
<th>Transferrin Saturation (%)</th>
<th>Serum Ferritin (µg/L)</th>
<th>HPcsi (µmol/L)</th>
<th>HIC (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys282Tyr/Cys282Tyr</td>
<td>11/8</td>
<td>37.6 ± 4.1</td>
<td>73 ± 5</td>
<td>810 ± 140</td>
<td>2.6 ± 0.2</td>
<td>68 ± 12</td>
</tr>
<tr>
<td>n = 19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cys282Tyr/WT</td>
<td>9/3</td>
<td>23.1 ± 2.9</td>
<td>40 ± 5</td>
<td>449 ± 86</td>
<td>1.8 ± 0.2</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>n = 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cys282Tyr/His63Asp</td>
<td>2/2</td>
<td>27.4 ± 1.8</td>
<td>52 ± 2</td>
<td>264 ± 160</td>
<td>2.0 ± 0.6</td>
<td>24 ± 13</td>
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<tr>
<td>n = 4</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>His63Asp/His63Asp</td>
<td>2/4</td>
<td>26.0 ± 4.3</td>
<td>48 ± 7</td>
<td>263 ± 67</td>
<td>1.8 ± 0.3</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>n = 6</td>
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<td></td>
</tr>
<tr>
<td>His63Asp/WT</td>
<td>8/4</td>
<td>23.0 ± 2.9</td>
<td>38 ± 6</td>
<td>414 ± 72</td>
<td>1.9 ± 0.3</td>
<td>40 ± 9</td>
</tr>
<tr>
<td>n = 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT/WT</td>
<td>20/8</td>
<td>21.3 ± 2.1</td>
<td>39 ± 4</td>
<td>447 ± 92</td>
<td>1.8 ± 0.2</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>n = 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>65/34</td>
<td>18.4 ± 0.7</td>
<td>29 ± 1</td>
<td>75 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 99</td>
<td></td>
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</tbody>
</table>

Normal HPcsi values are 0-1.20 Normal HIC values are <25 µmol/g dry weight. Data were derived from 81 genotyped patients who had not been treated by phlebotomy prior to evaluation. Values are the mean ± SEM, and median values are shown in parenthesis. Mean values for transferrin saturation, serum ferritin, and HIC for the Cys282Tyr/Cys282Tyr genotype were higher than values for all other genotypes (P = .0001, .0096, and .0004, respectively). The controls are members of hemochromatosis pedigrees sharing no HLA alloantigen with the proband. They were matched by sex and age with PCT patients.19

Table 4. Liver biopsy findings in 84 patients with PCT

<table>
<thead>
<tr>
<th>Stage</th>
<th>HCV+</th>
<th>HCV−</th>
<th>Not Tested for HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>NIF</td>
<td>HPCSI</td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>10</td>
<td>1.8</td>
</tr>
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<td>1</td>
<td>7</td>
<td>6</td>
<td>1.7</td>
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<td>2</td>
<td>14</td>
<td>14</td>
<td>1.8</td>
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<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2.6</td>
</tr>
</tbody>
</table>

NIF indicates the number of patients with a necroinflammatory process of grade 1 or higher. Mean HPCSI scores range from 0-4.21 EIOH indicates the mean score of alcohol consumption by grade. Grades range from 0-3, as described in “Materials and Methods.” Anti-HCV IgG was serologically detected by ELISA, as described in “Materials and Methods.” Histologic grading and staging of HCV was performed by the method of Batts and Ludwig.20 Grading is a measure of the severity of the necroinflammatory process, with grade 0 indicating normal. Staging refers to the degree of fibrosis present, with stage 0 indicating normal.
Table 5. Risk factors in Cys282Tyr homozygotes with PCT compared to Cys282Tyr homozygotes without PCT

<table>
<thead>
<tr>
<th>Factor</th>
<th>With PCT</th>
<th>Without PCT</th>
<th>Odds Ratio</th>
<th>95% CLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>10/16</td>
<td>15/57</td>
<td>4.7</td>
<td>1.5-14.4</td>
</tr>
<tr>
<td>HCV</td>
<td>6/14</td>
<td>3/60</td>
<td>14.3</td>
<td>3.7-54.6</td>
</tr>
<tr>
<td>Estrogen</td>
<td>6/7</td>
<td>9/27</td>
<td>12.0</td>
<td>1.6-87.6</td>
</tr>
</tbody>
</table>

Cys282Tyr homozygote controls without PCT were matched by sex and age (±5 years). All controls were adults. (One female with PCT was a prepuberal 12-year-old girl, and 3 Cys282Tyr homozygotes with PCT were children. They are not included here.) We matched 3 nonporphyric homozygotes for each homozygote with PCT. The alcohol study includes all subjects consuming more than 20 g of alcohol daily.

compared to matched controls without HCV, and the odds ratio for estrogen as a risk factor remained high at 9.0 (95% CLs, 1.1-71).

The analysis was expanded to PCT patients of all HFE genotypes, and again HCV and alcohol were highly associated. Of the PCT patients with HCV, 34/36 men and 6/6 women reported grade 2-3 alcohol consumption. Only 9 PCT patients with grade 2-3 alcohol consumption did not have HCV; 2 were Cys282Tyr homozygotes, 2 had F-PCT, and 1 had hepatitis B virus. In this study, 8 women without HCV and with only grade 0-1 alcohol consumption were taking estrogens, thereby confirming estrogen use as an independent risk factor. Only 1/108 PCT patients we evaluated, a 5-year-old girl with S-PCT, had no risk factors and was genetically normal at the URO-D locus.

Most patients with PCT had multiple risk factors; 80% of the men had more than 1 risk factor, and the most common combination was alcohol and HCV, which was found in 71% of these patients. Single risk factors were more common (40%) in women because of the effect of estrogen use. In 28% of women with PCT, estrogen was the only risk factor identified, and in 12% of the women, either alcohol or the Cys282Tyr/Cys282Tyr genotype was the only risk factor identified. More than 1 risk factor was found in the remaining 60% of women with PCT, and the most frequent combination was alcohol and HCV. This combination was found in 28% of the PCT women we studied.

**Discussion**

Mutations at the HFE locus, HCV infection, excess alcohol intake, and exposure to estrogens all proved to be risk factors for the development of PCT. HFE mutations and HCV infection imparted the greatest relative risk, and the presence of multiple risk factors was more frequent than the presence of single risk factors.

Hepatic iron overload is a nearly constant finding in patients with PCT, and iron depletion is the mechanism underlying the beneficial effect of phlebotomy therapy. Most patients with clinically evident hemochromatosis are homozygous for the ancestral Cys282Tyr mutation at the HFE locus, and it has been estimated that approximately half of the Cys282Tyr homozygotes develop clinically significant iron overload. Heterozygotes develop laboratory evidence of iron overload in about 20% of cases. A second mutation, designated His63Asp, is found in the heterozygous state in approximately 20% of the Caucasian population. The clinical consequences of the His63Asp mutation are probably minor, although compound heterozygosity for the 2 mutations (genotype Cys282Tyr/His63Asp) has a greater effect on body iron burden than simple heterozygosity for the Cys282Tyr mutation (genotype Cys282Tyr/WT).

Mutant HFE alleles were detected in 63% of the PCT patients we genotyped (Table 2). Homozygosity for the Cys282Tyr mutation was found in 19%, and an additional 15% were simple heterozygotes (genotype Cys282Tyr/WT). These results confirm the report of Roberts et al., who found that 17% of 41 British patients were Cys282Tyr homozygotes and 20% were heterozygotes. Similar findings were also reported by Bonkovsky et al in 26 American PCT patients. Collectively, these data suggest that the Cys282Tyr/WT genotype is not overrepresented in patients with PCT. All of our patients with PCT had an iron phenotype that differed significantly from nonporphyric controls (Table 3), but only Cys282Tyr homozygotes had an iron phenotype different from PCT patients with any other HFE genotype. These data indicate that the most dramatic effect of HFE mutations on the hepatic iron overload seen in PCT occurs when the Cys282Tyr/Cys282Tyr genotype is present.

We found 6 patients with the Cys282Tyr/His63Asp genotype, but we could not confirm an effect on the iron phenotype (Table 3). In a study of Italian men with PCT, Sampietro et al found heterozygosity for the His63Asp mutation (genotype His63Asp/WT) more often than in controls, but the mutation was not related to iron status. The His63Asp/WT genotype was not over represented in our PCT patients (Table 2), and we also found no impact of this genotype on the iron phenotype (Table 3).

The mechanism responsible for liver iron loading in PCT patients without a Cys282Tyr or His63Asp mutation has not been defined. Other HFE mutations associated with iron overload have recently been described, but these occur infrequently. The HFE gene product regulates cellular iron metabolism through an interaction with the membrane-bound transferrin receptor, but whether this interaction enhances or diminishes delivery of iron to cells remains unresolved.

Both HCV infection and alcohol excess have been associated with hepatic siderosis. We found no significant difference between the frequency of HCV infections in PCT patients with mutant HFE alleles and those with WT alleles, which suggests that HFE mutations do not predispose to HCV infections. A similar conclusion was reached by Smith et al in a study of 137 British patients with chronic HCV infections. Our data, however, clearly indicate that HCV infection predisposes to the development of PCT, confirming earlier reports. Approximately 75% of patients with PCT in the United States, Italy, Spain, and France have laboratory evidence of HCV exposure, but the HCV association is less common in patients with PCT from Northern Europe, Australia, and New Zealand.

An increase in the serum iron concentration, the percent of saturation of transferrin, and the serum ferritin concentration occurs commonly in patients with HCV, although hepatic siderosis is a less common occurrence. When the serum ferritin and hepatic iron concentrations are elevated, HCV patients are less likely to respond to therapy with α interferon (IFN-α). It remains unknown, however, if infection with HCV leads to hepatic iron excess or if preexisting siderosis enhances the damaging effects of the virus. All of our patients with PCT and HCV responded to phlebotomy therapy, but IFN-α therapy has proven useful in the rare instance when phlebotomy therapy has failed.

We found the most severe changes in liver biopsy samples from PCT patients with HCV who were iron loaded and consumed more than 70 g of alcohol daily. Many reports have indicated that alcohol accelerates the development of hepatic fibrosis in patients with HCV. The association between excess alcohol consumption and PCT is well recognized, but we found it difficult to evaluate alcohol as an independent risk factor because both alcohol abuse...
and HCV were found in 71% of our male patients and 28% of our female patients. Animal studies have demonstrated that alcohol intake causes hepatic iron deposition, and it has been estimated that up to one-third of alcoholics develop hepatic iron overload. LeSage et al concluded that alcohol-associated iron overload was likely due to the effects of human leukocyte antigen–associated (HLA-associated) hemochromatosis, and Simon et al confirmed this conclusion. Our data suggest that alcohol is associated with hepatic iron loading in patients with PCT, but this effect is magnified by homozygosity for the Cys282Tyr HFE mutation. Phlebotomy therapy is effective in treating PCT in alcoholics, even if excessive alcohol intake continues throughout therapy. This strongly suggests that iron is the key etiologic factor.

Approximately 18% of American women between the ages of 18 and 44 use oral contraceptives, and 15% of postmenopausal American women use medicinal estrogens as hormone replacement therapy. Of the women with PCT in our study, 63% were ingesting estrogen preparations at the time that PCT became clinically apparent, and in 28% of these women, estrogen was the only precipitating factor identified. Estrogen proved to be an independent risk factor for the development of PCT, but the mechanism responsible for the estrogen effect is unknown. Estrogens, but this occurs only when exposure to estrogens has not been prolonged.

The activity of hepatic URO-D is reduced in both F-PCT and S-PCT. Half-normal activity of hepatic URO-D in heterozygotes for URO-D mutations is not rate limiting, as most carriers of mutant alleles of URO-D do not express a clinical phenotype. In S-PCT hepatic URO-D, protein concentration is normal even though enzyme activity is reduced. Our data indicate that the same risk factors (iron excess, alcohol, HCV, and estrogen) contribute to clinical expression of both types of PCT, which suggests that 1 or more of these factors contribute to the generation of a liver-specific inhibitor of URO-D. Compounds that induce specific isoforms of P450 can induce PCT in both humans and rodents.

A liver-specific isozyme of cytochrome P450, P4501A2, is essential for chemically-induced porphyria in mice, but induction of CYP1A2 alone is not sufficient to produce a porphyric response in rats. These data indicate that CYP1A2 is required but not sufficient to produce the rodent porphyrin model.

In humans, expression of CYP1A2 is constitutive and highly variable and can be affected by smoking, drugs, cruciferous vegetables, and other factors. We sought immunohistochemical evidence for increased levels of CYP1A2 in hepatocytes of patients with PCT, but we found no correlation between the level of CYP1A2 and the expression of F-PCT or S-PCT (data not shown). These data, coupled with the findings in the CYP1A2 knockout mouse, suggest that some threshold level of CYP1A2 activity is central to the pathogenesis of PCT, but exceeding that threshold has no additional effects.

Both genetic and environmental factors contribute to the pathogenesis of PCT. Two genetic factors have been identified: mutations at the HFE locus and at the URO-D locus. The frequency of homozygosity for the Cys282Tyr HFE mutation is much greater than the frequency of PCT, which indicates that hemochromatosis alone cannot be responsible for the development of PCT. The frequency of URO-D mutations in the population is unknown, but it is unlikely that it is as high as the 29% incidence we found in our PCT population. In pedigrees with F-PCT, most individuals who carry mutant URO-D alleles do not express the porphyric phenotype, which indicates that mutant URO-D alleles alone cannot be responsible for the development of PCT. Unidentified genetic factors probably play a role in the pathogenesis of PCT, as even S-PCT may be familial. The proportion of the population exposed to excess alcohol, HCV, and medicinal estrogens is also far higher than the frequency of PCT, indicating that these risk factors alone do not cause PCT. An unclarified interaction between environmental and genetic factors appears to be required for phenotypic expression of PCT in most cases.

Approximately 20% of patients presenting with PCT are homozygous for the Cys282Tyr mutation at the HFE locus, and the majority of PCT patients have been exposed to HCV. These 2 risk factors should be examined in all newly diagnosed patients with PCT. Genotyping at the HFE locus is now widely available through commercial diagnostic laboratories. This method of detecting hemochromatosis homozygotes in association with PCT is preferred, as the iron phenotype is abnormal even in patients with PCT who do not have genetic hemochromatosis (Table 3). Family studies are warranted when a proband with PCT is found to be homozygous for the Cys282Tyr mutation. Iron depletion by repetitive phlebotomy is the preferred therapy for most patients with PCT and may improve the clinical course of HCV infection.

**References**

14. Lundvall O, Weinfeld A. Studies of the clinical and