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# Porphyria Cutanea Tarda, Hepatitis C, and HFE Gene Mutations in North America

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In some, but not all countries, porphyria cutanea tarda (PCT) has been associated with chronic infection with the hepatitis C virus (HCV). Recently, PCT has also been associated with mutations in the HFE gene that are associated with HLA-linked hereditary hemochromatosis. Until now, few studies of these associations have been reported from North America. The aims of this study were: 1) to assess the prevalence of HCV infection and HFE mutations in North American patients with PCT; 2) to compare demographic and laboratory features between those who are HCV-positive and HCV-negative; and 3) to study urinary porphyrin excretions in American HCV-positive patients without clinically manifest PCT. Clinical and laboratory data, including tests for HCV and urinary porphyrins, were collected from 70 unselected patients with typical PCT. Urinary porphyrins were also measured in 110 non-PCT patients with chronic hepatitis C. Mutational analyses of the HFE gene were performed in 26 PCT patients. Thirty-nine of 70 (56%) of the PCT patients had evidence of HCV infection. Thirty-two of 39 PCT patients with HCV were men, all of whom used alcohol. In contrast, 22 of 31 PCT patients without HCV infection were women, 12 of whom had taken estrogens. The HCV-positive group was more likely to have used illicit intravenous drugs (45% vs. 0%;  $P = 0.01$ ), to have had several ( $>4$ ) sex partners (48% vs. 13%;  $P = 0.005$ ), and less likely to have no known risk factors for HCV infection (33% vs. 78%;  $P = 0.004$ ). Total urinary porphyrin excretion was the same in the two groups, but those with HCV infection had a significantly lower percentage of uroporphyrin and higher percentages of hepta- and hexa-carboxy porphyrins in urine. Sixteen of 110

(15%) HCV-positive subjects without PCT had increased urinary porphyrins, but, unlike PCT, these were mainly coproporphyrin. Forty-two percent of PCT patients carried the C282Y mutation of HFE (15% homozygous), and another 31% carried the H63D mutation (8% homozygous). Thus, 73% of PCT patients had one of these mutations. The prevalence of HCV infection (56%) and mutations in the HFE gene (73%) are high among North American patients with PCT. Alcohol and estrogen use are important additional risk factors. All PCT patients should be tested for HCV infection and for HFE gene mutations. Although HCV infection is a trigger for PCT, preclinical PCT is rare in chronic HCV hepatitis C in the United States. (HEPATOLOGY 1998;27:1661-1669.)

Porphyria cutanea tarda (PCT)<sup>1</sup> is the most common form of porphyria. Usually, the presenting feature is a vesiculo-bullous eruption on the hands and face, which is caused by deposits of uro- and heptacarboxy-porphyrins in the skin that sensitize the photon-driven formation of singlet oxygen. In PCT, excess porphyrins are produced chiefly in the liver, because of a complex interplay of factors that may include: 1) decreased activity of hepatic uroporphyrinogen decarboxylase (Uro-D); 2) increased formation of uroporphyrinogen and other porphyrinogen substrates of Uro-D; and 3) increased oxidation of porphyrinogens to porphyrins, which are not substrates for Uro-D. In a minority (20%-25%) of subjects with PCT, there is an inherited partial defect in the activity of Uro-D. The activity is about 50% of normal, but, in itself, this is not sufficient to produce overt PCT. Additional factors are also necessary. Chief among these are underlying liver disease and iron in the liver (for reviews, see Bonkovsky,<sup>1</sup> Kappas,<sup>2</sup> and Elder<sup>3</sup>).

Hepatic injury is usually present in clinically overt PCT and was formerly ascribed chiefly to heavy alcohol use. However, recent studies in Spain, France, and Italy<sup>4-6</sup> indicated that a substantial majority (71%-91%; average, 77%) of subjects with PCT had evidence of infection with the hepatitis C virus (HCV). In contrast, the prevalence of HCV infection was low (0%-18%) in PCT patients from Northern or Central Europe,<sup>3,7-10</sup> Australia,<sup>11</sup> or New Zealand.<sup>12</sup> Until now, the relationship of HCV to PCT has been studied in few North American patients.<sup>13,14</sup> It is not known whether HCV predisposes to PCT or vice versa.

A potential link between PCT and chronic hepatitis C is iron in the liver. It has been known for many years that: 1) most patients with PCT have some degree of iron overload; 2) iron removal ameliorates porphyrin overproduction and clinical features; and 3) administration of iron produces

Abbreviations: PCT, porphyria cutanea tarda; Uro-D, uroporphyrinogen decarboxylase; HCV, hepatitis C virus; HHL, HLA-linked hereditary hemochromatosis; PCR, polymerase chain reaction; HIV, human immunodeficiency virus; GGT,  $\gamma$ -glutamyl transpeptidase.

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relapse.<sup>1-3</sup> Recently, 44% to 47% of PCT patients from the United Kingdom<sup>15</sup> or the Netherlands<sup>16</sup> were reported to carry the C282Y mutation in the HFE gene that is responsible for the great majority of HLA-linked hereditary hemochromatosis.<sup>17</sup> Iron may act to increase production of uroporphyrinogen, to increase the likelihood of its oxidation, and to decrease activity of Uro-D.<sup>1-3,19,20</sup> An association between ferritin and crystals of uroporphyrin has been noted in livers of patients with PCT.<sup>21</sup> A role for iron in affecting severity and response to therapy of chronic hepatitis B and C has also been recognized (for reviews, see Bonkovsky<sup>22-24</sup>). Iron reduction may improve the severity of chronic hepatitis C and increase the likelihood of response of chronic hepatitis C to antiviral therapy.<sup>22-24</sup>

The aims of this work were threefold: 1) to determine the prevalence of HCV infection and the HFE mutations associated with HHC in PCT patients from North America; 2) to learn whether there are demographic, clinical, or biochemical differences between such PCT patients with or without HCV infection; and 3) to see whether patients with chronic hepatitis C without clinical PCT have abnormal urinary porphyrin profiles typical of PCT. A portion of this work has been presented in abstract form.<sup>25</sup>

## PATIENTS AND METHODS

**Patients With PCT.** Data on 70 unselected patients initially evaluated for the disease between 1992 and 1996 were collected and summarized. All of these patients were seen principally for evaluation and management of their skin disease; most were seen at a few centers recognized for studies of porphyrias (e.g., Centers at Case-Western Reserve, Columbia, New York Medical College, University of California, Davis, University of Massachusetts), although some were contributed by other colleagues (see Acknowledgment). None of the patients were referred principally because of known or suspected liver disease. Diagnosis of PCT was based on a typical constellation of clinical and laboratory features. All patients had marked increases in uro- and heptacarboxy-porphyrins. When tested, urinary porphobilinogen was uniformly normal, and 5-aminolevulinic acid was normal or only slightly increased. Stool porphyrins were normal or slightly increased in coproporphyrin, presumably because of increased iso-coproporphyrin.

All patients had tests for antibodies to HCV (EIA II and 4-antigen strip immunoblot) and/or HCV RNA by branched DNA assay or by assays that use the polymerase chain reaction (PCR) for amplification of viral RNA. Those positive for antibodies and/or RNA were considered to have been infected with HCV. Erythrocytic activity of Uro-D was measured in 31 patients, and mutations of the HFE gene were sought in 26 patients, from whom samples were available for testing.

**Patients With Chronic Hepatitis C Without Clinically Manifest PCT.** Two groups of patients were studied: 1) 95 unselected patients with known chronic hepatitis C from whom urines were obtained for measurement of porphyrins, and 2) 15 patients with no personal or family history of porphyria, who were age- and sex-matched to 15 of the patients with PCT and known chronic HCV infection.

**Methods.** Urinary porphyrins and porphyrin precursors (5-aminolevulinic acid and porphobilinogen) were measured as described previously.<sup>25</sup> Urines were stored frozen ( $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$ ) until assayed. Standard blood tests and serological tests for hepatitis B virus and HCV were performed in the hospital laboratories in which the patients were seen. Detection of HCV RNA was performed by the bDNA assay (Chiron, Emeryville, CA) or by PCR.<sup>26</sup> Genotyping of HCV was performed by the National Genetics Institute (Culver City, CA).<sup>27</sup>

Activity of Uro-D was measured in crude or partially purified erythrocytic lysates, using pentaporphyrinogen I as substrate.<sup>28</sup> The

product coproporphyrinogen was oxidized to coproporphyrin and quantified by high-performance liquid chromatography (HPLC).<sup>29</sup> With this assay, the mean (range) activity for normals is 3.5 nmol coproporphyrinogen I  $\cdot$  h<sup>-1</sup>  $\cdot$  mg protein<sup>-1</sup> (2.7-4.5), and for familial (type II) PCT, it was 1.5 nmol coproporphyrinogen I  $\cdot$  h<sup>-1</sup>  $\cdot$  mg protein<sup>-1</sup> (0.9-2.4).

Needle liver biopsies were processed by standard techniques, sectioned, and stained with hematoxylin-eosin, Masson's trichrome, and Perls' Prussian blue stains. They were evaluated for the presence of fibrosis, necrosis, and inflammation,<sup>30</sup> and for the presence or absence of fat and iron. When iron was present, the degree of positivity was assessed semiquantitatively.<sup>31</sup>

Mutational analysis of the HFE gene was performed on DNA extracted from frozen stored samples of blood using the QIAamp Blood Kit (Qiagen, Santa Clarita, CA) and amplified by PCR, which was followed by restriction endonuclease digestion. The products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. For both the C282Y and the H63D mutations, known homozygous normal, heterozygous, and homozygous abnormal controls were run concurrently with each batch of unknowns. The primers used for PCR were as follows: for C282Y, 5'-TGG CAA GGG TAA ACA GAT CC-3' and 5'-CTC AGG CAC TCC TCT CAA CC-3'; for H63D, 5'-ACA TGG TTA AGG CCT GTT GC 3' and 5'-GCC ACA TCT GGC TTG AAA TT-3'.<sup>17</sup> The restriction enzymes used were *SnaBI* for C282Y and *BclI* for H63D. The conditions for running the PCR were as described,<sup>32</sup> except that the annealing step was changed to 62°C for 30 seconds.

**Data Analysis.** Data were analyzed by both parametric (ANOVA,  $\chi^2$ ) and nonparametric (Wilcoxon/Kruskal-Wallis, rank sum, and median) tests.  $P < 0.05$  was considered significant. Analyses were aided by JMP 3.02 statistical software (SAS Institute, Cary, NC).

## RESULTS

**Demographic Features and Risk Factors for Disease.** Forty male and 30 female patients with PCT were studied. Their ages ranged from 14 to 73 years, with a mean age (SD) of 47.6 (11.8) years. Thirty-nine (56%) were positive for HCV. The mean ages of those who were positive for HCV infection were significantly lower than for those who were negative (Table 1).

A significantly higher proportion of those positive for HCV infection were male and gave a history of alcohol use (Table 1). In contrast, those with PCT who were negative for HCV were more likely to be women and to have used estrogens. With one exception, estrogen users were women who took them for contraception. A 44-year-old man who had taken estrogens was also HCV-positive. The prevalence of a mutation in the HFE gene was higher in PCT patients without HCV infection, although the difference was not significant (Table 1 and see below).

Risk factors for HCV infection, particularly intravenous drug use and multiple sex partners (more than four in the decade before diagnosis), were significantly more frequent in PCT patients who were HCV-positive (Table 1).

**Urinary Porphyrins and Erythrocytic Uro-D Activities.** Total urinary porphyrin excretions were markedly increased in all patients with clinically active PCT. For the entire group, the mean was 4,300, the median was 3,000, and the 25th to 75th percentiles was 1,760 to 4,720  $\mu\text{g}/24$  h. Total urinary porphyrin excretions did not differ between those who were HCV-positive or HCV-negative (Fig. 1A). However, those who were HCV-positive excreted a significantly lower percentage of total urinary porphyrin as uroporphyrin (59%) than those who were HCV-negative (67%) ( $P = 0.03$  by the Student's *t* and rank sum tests). The HCV-positive PCT

TABLE 1. Demographic Features and Risk Factors for Disease of Patients Studied

	PCT+ HCV−	PCT+ HCV+	PCT− HCV+
No. of subjects	31	39	15
Gender (M/F)	8/23*	32/7	11/4
Age (yr)			
Mean	51†	45	49
SD	14.3	8.2	11
25-75 percentile	45.5-61	39-49.5	39-53
Range	14-73	31-71	35-73
Duration of PCT (mo) at time of study			
Mean	12.8	12.5	N/A
SD	12.4	10.1	N/A
25-75 percentile	5-13	5-18.5	N/A
Range	1-51	1-46	N/A
Risk factors for PCT			
Alcohol use	15/30 (50%)*	33/34 (97%)	6/12 (50%)†
Estrogen use	12/27 (44%)*	2/33 (6%)	0/11 (0%)
Familial PCT	4/28 (14%)	10/33 (30%)	N/A
HFE gene mutations	10/14 (71%)	9/12 (75%)	ND
Risk factors for HCV			
IVDU	0/24 (0%)*	15/33 (45%)	10/15 (67%)
Blood transfusion	0/23 (0%)	1/33 (3%)	4/15 (27%)
Multiple sex partners	5/24 (21%)‡	16/33 (48%)	1/15 (7%)†
Several risk factors	0/23 (0%)†	10/32 (31%)	1/15 (7%)
No known risk factors	18/23 (78%)‡	11/32 (34%)	2/15 (13%)

Abbreviations: F female; IVDU, intravenous drug use; M, male; N/A, not applicable; ND, not determined.

\*Differs from PCT+, HCV+,  $P < 0.001$ .

†Differs from PCT+, HCV+,  $P < 0.05$ .

‡Differs from PCT+, HCV+,  $P < 0.005$ .

patients excreted significantly higher percentages of hepta- and hexa-carboxy porphyrins (Fig. 1B). Activities of erythrocytic Uro-D did not differ significantly between the two groups. Four of 28 (14%) and 10 of 33 (30%) patients with PCT who were HCV-negative and HCV-positive, respectively, had reduced erythrocytic Uro-D activities (considered evidence of an inherited predisposition to develop PCT). The

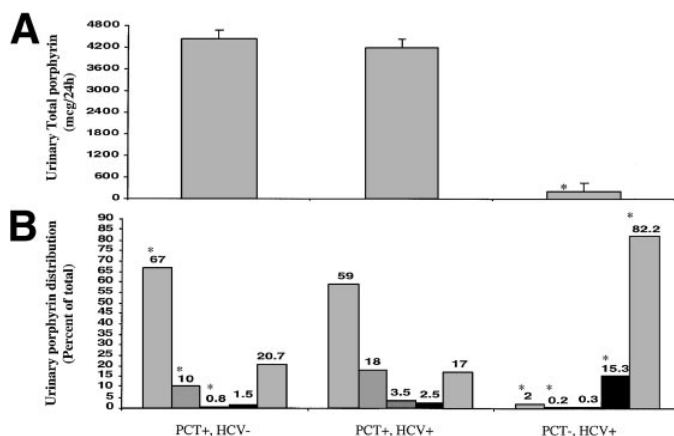


FIG. 1. Urinary porphyrins in subjects studied. Porphyrins were assayed as described in Patients and Methods. Asterisks denote values that differed from PCT+, HCV+ ( $P < 0.05$ ). The reference ranges are as follows: total urinary porphyrins (A)  $< 350$  g/24 h; porphyrin profile (B), each set of bars from left to right: 8-COOH (uroporphyrin)  $< 20\%$ ; 7-COOH,  $< 8\%$ ; 6-COOH,  $< 5\%$ ; 5-COOH,  $< 3\%$ ; 4-COOH (coproporphyrin)  $> 80\%$ . Results are mean  $\pm$  SE.

difference between these two prevalences was not significant ( $P = 0.22$ , Fisher's Exact two-tail test).

**Mutational Analyses of the HFE gene in Subjects With PCT.** We were able to test for the presence of the C282Y and H63D imitations of the HFE gene in 26 of our patients with PCT. As shown in Table 2, there were strikingly high frequencies of both mutations: 11 of 26 (42%) carried the C282Y mutation (23% heterozygous and 19% homozygous), and 8 of 26 (31%) carried the H63D mutation (23% heterozygous and 8% homozygous). No patient carried both mutations. Nineteen of 26 (73%) subjects studied had a defined mutation in the HFE gene that has been associated with iron overload.

Those who were homozygous for the C282Y mutation had significantly higher serum iron concentrations ( $200 \pm 11$ ) and transferrin saturations ( $73 \pm 5$ ) than those who were normal ( $121 \pm 12$  and  $40 \pm 4$ ) or heterozygous ( $67 \pm 9$  and  $29 \pm 5$ ) (Table 3). Surprisingly, heterozygotes had lower values than normals for these variables. Mean serum ferritins were increased in all groups, with no significant differences among the groups. Both of the homozygote abnormal patients who had liver biopsies showed 4+ iron staining, whereas four of six normals and the one heterozygote biopsied had 0-1+ iron staining ( $P = 0.03$  by Pearson's test) (Table 3). There were no differences in measures of iron metabolism among those with or without the H63D mutation (Table 3).

Values for other laboratory variables such as serum transaminases, alkaline phosphatase,  $\gamma$ -glutamyl transpeptidase (GGT), albumin, bilirubin, and total protein were not different among subjects who were normal, heterozygous, or homozygous abnormal for the two HFE gene mutations. Other than iron staining, hepatic histopathological findings were not different among these groups (data not shown).

Four of the 11 patients who carried the C282Y mutation (including two of five homozygotes) were HCV-positive, whereas 7 of 11 were negative ( $P = 0.29$ ). Six of the 8 who carried the H63D mutation (including 1 of 2 homozygotes) were HCV-positive ( $P = 0.22$ ).

**Values of Selected Blood Tests: Relationship to HCV Status.** Among several blood tests, including complete blood counts, liver chemistries (Fig. 2), measures of iron status (Fig. 3), and tests of blood coagulation, there were significant differences between HCV-positive and HCV-negative PCT patients only in hemoglobin concentrations and leukocyte counts. The difference in hemoglobin concentrations was attributable to the difference in sex distributions between the two groups (Table 1). Increases in serum ferritin and transferrin saturations were frequent and equally common in those without as with HCV infection, and mean values of these measures of iron status did not differ among the groups (Fig. 3). As shown in Table 4, at baseline evaluation, men with chronic hepatitis C were particularly likely to have increases in serum transferrin saturation (overall 11 of 34 [32%]). Both men and women

TABLE 2. Results of HFE Mutational Analysis in 26 Subjects With PCT

Genotype	Amino Acid Position	
	282	63
Wild-type normal	15 (58%)	18 (69%)
Heterozygous	6 (23%)	6 (23%)
Homozygous abnormal	5 (19%)	2 (8%)

NOTE. Mutational analysis were performed by PCR-RFLP as described in Patients and Methods.

TABLE 3. Measures of Iron Status in Relation to HFE Gene Mutations in 26 Subjects With PCT

Variable Measured	Mutation					
	C282Y			H63D		
	-/- NI	+/- Hetero	+/+ Homo Abnl	-/- NI	+/- Hetero	+/+ Homo Abnl
Serum iron (mcg/dL)	121 ± 12	67 ± 9*	200 ± 11*	128 ± 16	122 ± 9	88 ± 23
Serum IBC (mcg/dL)	317 ± 11	277 ± 20	275 ± 16	300 ± 12	316 ± 22	290 ± 11
Serum Tf saturation (%)	40 ± 4	29 ± 5*	73 ± 5*	49 ± 6	40 ± 6	31 ± 9
Serum ferritin (ng/mL)	325 ± 76	452 ± 130	318 ± 86	367 ± 72	343 ± 121	197 ± 30
Hepatic iron staining (0-4+)	1-0+ 3-1+ 2-3+	1-1+	2-4+	1-0+ 1-1+ 2-3+	2-1+	1-0+

NOTE. Results of serum variables are mean ± SE.

Abbreviations: Abnl, abnormal; Hetero, heterozygous; Homo, homozygous; n, number of subjects; NI, normal.

\*Differs from normal ( $P < 0.05$ ).

with PCT, with or without chronic hepatitis C, were likely to have increased serum ferritins (19 of 32 [59%] men and 14 of 21 [67%] women), whereas only 1 of 4 (25%) women, but 7 of 11 (64%) men without PCT but with chronic hepatitis C had increased serum ferritin ( $\chi^2 = 0.12$ ; Fisher's two-tailed  $P = 0.27$ ).

**Iron Removal as Therapy for PCT.** Abstinence from alcohol and therapeutic phlebotomy were recommended as the initial treatment of choice for PCT, irrespective of patients' HCV status. (HFE mutational studies were all performed retrospectively, 1 year or more after patients had initially been seen). We were able to obtain data on the amounts of iron removed to achieve initial amelioration of PCT in 19 subjects (13 men [11 HCV-positive] and 6 women [all HCV-negative]). A mean of 13.85 g of iron was removed from the men (range, 6-24 g; median, 12 g) and a mean of 7.3 g from the women (range, 2-16 g; median, 5.25 g). The difference between men and women was significant ( $P = 0.04$ ). Significantly more iron was removed from the 11 HCV-positive subjects than the 8

HCV-negative subjects (mean values, respectively, 14.9 vs. 7.4 g;  $P = 0.05$ ). We obtained maintenance therapeutic venesection data on 9 HCV-positive men and 5 HCV-negative subjects (2 men, 3 women). The amount of iron removed annually ranged from 0.38 to 5.0 g. Perhaps because of the small numbers of subjects, differences between men and women and/or HCV-positive and HCV-negative subjects were not significant.

The amount of iron removed initially and during maintenance was highest in those homozygous for the C282Y mutation, intermediate for heterozygotes, and lowest for homozygous normal subjects. However, numbers of subjects for whom these data were available were small, and differences were not significant. We had no iron-removal data available for those homozygous for the H63D mutation; heterozygotes for this mutation were not different from homozygous normals (wild type).

**Comparison of HCV-Positive Patients With and Without PCT.** Comparison of an age- and sex-matched control group of

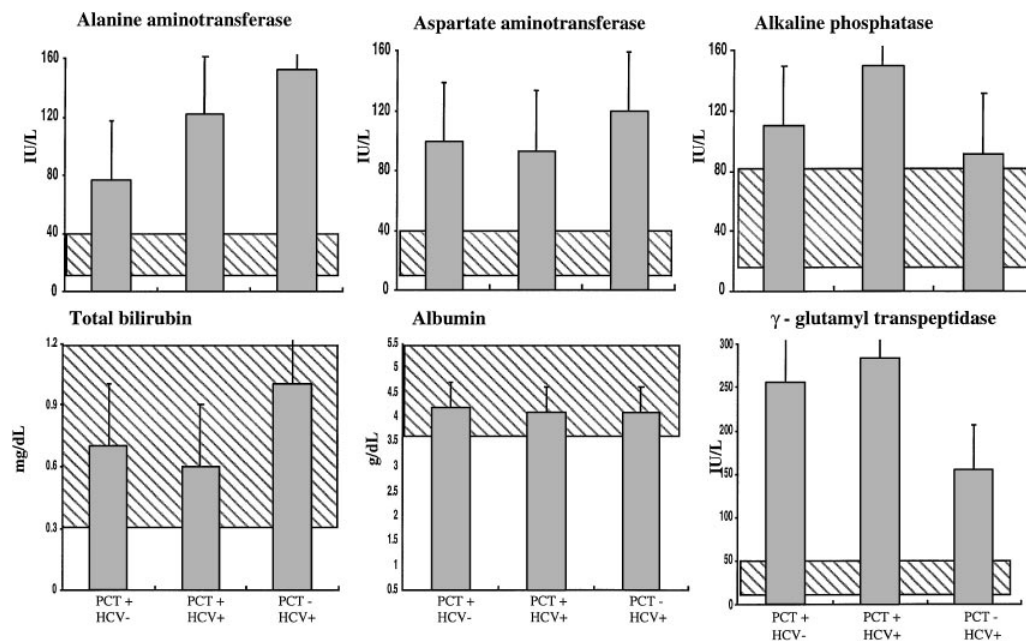


FIG. 2. Selected liver chemistries in subjects studied. Tests were performed in the clinical laboratories of the medical centers where the patients were seen. Results are presented as in Fig. 1. The reference ranges for the analytes are shown (▨). None of the differences among groups are significant.

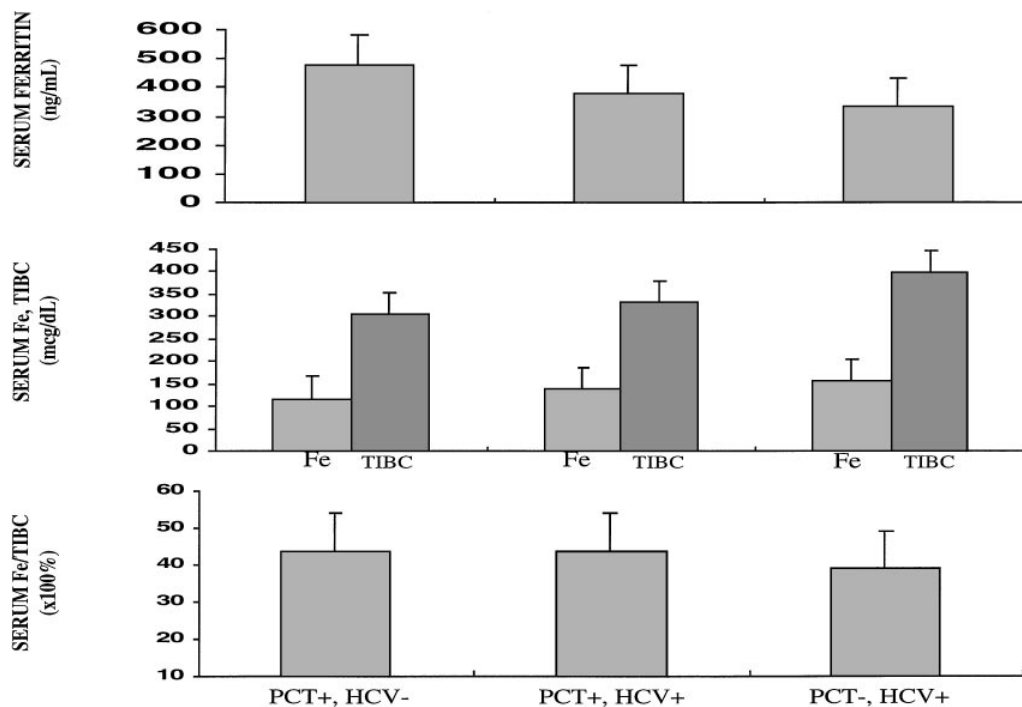


FIG. 3. Measures of iron status in subjects studied. Results are presented as in Fig. 1. The reference ranges for the analytes are as follows: serum ferritin, for men: 12-283 ng/mL; for women: 12-150 ng/mL; serum iron, 76-198  $\mu$ g/dL; TIBC, 255-450  $\mu$ g/dL; serum Fe/TIBC ( $\times 100\%$ ) 20%-50%. None of the differences among groups is significant. Fe, iron; TIBC, total iron-binding capacity.

HCV-positive patients without PCT to those who were HCV-positive and had clinically active PCT revealed relatively few differences. Indeed, except for urinary porphyrins (Fig. 1), results of laboratory tests showed generally similar results (Figs. 2 and 3). Those without PCT had higher values for total iron-binding capacity, prothrombin time, and partial thromboplastin time of borderline statistical significance. Those without PCT also were significantly less likely to be alcohol users or to have had many sex partners (Table 1).

We studied with particular care serum levels of transaminases and GGT in relation to HCV infection and alcohol use. Mean values of serum alanine transaminase and aspartate transaminase were higher in alcohol users than nonusers, regardless of HCV status, whereas, surprisingly, the mean GGT level was higher in HCV-negative, alcohol nonusers than in other groups (Table 5). However, ranges were broad,

and numbers of subjects, especially those abstaining from alcohol, were small. None of the differences was significant.

**Urinary Porphyrins in Unselected Patients With Chronic Hepatitis Without Evidence of PCT.** Among 110 patients tested, the prevalence of increased total urinary porphyrin concentration (defined as  $>125 \mu$ g/L) was 15% (16 of 110), and the prevalence of abnormally increased fraction of total porphyrin as uro- + heptacarboxy-porphyrins (defined as  $>20\%$ ) was 4.5% (5 of 110).

**Viral Genotypes in Subjects Studied.** Viral genotyping was performed successfully on 7 patients with PCT and HCV and 11 patients with HCV without PCT. Six patients with PCT

TABLE 4. Prevalence of Increased Serum Transferrin Saturation and Ferritin in Subjects Studied

Variable Measured	Patient Group		
	PCT+, HCV-	PCT+, HCV+	PCT-, HCV+
Serum Transferrin Saturation $>50\%$	No. increased/total no. (percent)		
All subjects	4/24 (17%)	9/28 (32%)	3/7 (43%)
Men	2/10 (20%)	9/24 (38%)	3/5 (60%)
Women	2/14 (14%)	0/4 (0%)	0/2 (0%)
Serum Ferritin $>ULN$	No. increased/total no. (percent)		
All subjects	12/21 (57%)	21/32 (66%)	8/15 (53%)
Men	3/6 (50%)	16/26 (62%)	7/11 (64%)
Women	9/15 (60%)	5/6 (83%)	1/4 (25%)

NOTE. None of the differences among groups is significant.

TABLE 5. Values of Serum Transaminases and GGT in PCT Subjects Studied: Influences of HCV Infection and Alcohol Use

Variable Measured	Alcohol Users		Alcohol Nonusers	
	HCV-	HCV+	HCV-	HCV+
ALT				
n	12	27	4	1
Mean	82	112	33	39
SE	12.4	24.4	7.5	—
AST				
n	13	30	6	0
Mean	90	97	39	—
SE	36.9	19.7	6.9	—
GGTP				
n	10	22	4	0
Mean	179	235	278	—
SE	65.8	47.8	197	—

NOTE. None of the differences within or between groups is significant.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; n, number of subjects studied.

were genotype 1a and 1 was 1b. In those without PCT, HCV genotypes were as follows: 4 were 1a; 4 were 1a/1b, mixed; and 1 each was 1b, 2b, and 3a.

**Hepatic Histopathology in Subjects Studied.** Results of liver biopsies were available for 20 of 70 patients with PCT (16 HCV-positive) and 13 of 15 PCT-negative patients with chronic hepatitis C. On the whole, the biopsies showed mild to moderate fibrosis, fatty change, iron deposition, hepatocyte necrosis, piecemeal necrosis, and lympho-histiocytic infiltration of portal tracts, in keeping with previous reports of changes in PCT<sup>1-3,20</sup> or chronic hepatitis C.<sup>33,34</sup> Seven of the 16 HCV-positive PCT patients had moderate (2-3+) fibrosis, and none had cirrhosis (4+ fibrosis), whereas none of the 4 PCT patients who were HCV-negative had moderate fibrosis (2 had 0 and 2 had 1+ fibrosis). Two patients in each group had heavy (3-4+) iron staining. Perhaps because of the relatively small numbers of patients available for study, especially in the HCV-negative group, these differences were not statistically significant ( $P = 0.26$  and  $0.09$ , respectively). As shown in Table 3, 2 of the 4 were homozygous for the HFE C282Y mutation, whereas the other 2 had neither the C282Y nor the H63D mutation. There were also no differences among the groups in degree of fatty change or inflammation. Comparison of hepatic histopathology of HCV-positive patients with and without PCT also showed no significant differences in fibrosis scores or iron scores (fibrosis was 2+ in 7 of 16 with PCT and 8 of 13 without PCT, and iron was 2+ in 4 of 12 with PCT and 3 of 13 without PCT.) The only significant difference in histopathological findings was significantly more hepatocytic necrosis in those who were HCV-positive (regardless of PCT status) than in those who were HCV-negative ( $P = 0.015$  by Pearson's test).

## DISCUSSION

Recent studies, particularly from Europe (summarized in Table 6), report wide variations in the prevalence of HCV infection in patients with active PCT. The prevalence is high (70%-90%) in Southern Europe (France, Italy, Spain) but low (0%-18%) in Northern Europe, Australia, and New Zealand (Table 4). Until now, few North American PCT patients have been reported.<sup>13,14</sup> Our results show a prevalence of HCV infection among North American PCT patients (56%) that is between these extremes, although closer to those of Southern Europe. Our patients were mainly whites of European extraction. We were not able to obtain detailed family histories, so we cannot be sure where their ancestors came from nor for how many generations they had been in the United States.

We identified risk factors for PCT (other than HCV infection) in all our patients, the most common being a history of alcohol use and HFE gene mutations (Tables 1 and 2). We did not attempt to subclassify patients according to amounts of admitted alcohol use, because this is often erroneously low and because, in the presence of liver disease (e.g., due to HCV or iron loading), even small amounts of alcohol may be injurious.<sup>22,23</sup> The higher prevalence of familial PCT in our study compared with those of others (Tables 1 and 6), is likely because we defined familial PCT based on activities of erythrocytic Uro-D, not on family history alone. Such a definition is preferred, because most subjects with an inherited heterozygous defect in the Uro-D gene do not develop overt PCT.<sup>1-3</sup> The prevalence of familial PCT that we found is similar to the approximately 25% found by others, based on Uro-D activities.<sup>3,39</sup>

What is the link between PCT and chronic hepatitis C? On grounds of logic, three possible answers to this question may be suggested: 1) PCT predisposes to development of HCV infection; 2) chronic HCV infection predisposes to development of PCT; and 3) other factors, independent of both PCT and chronic hepatitis C, predispose to development of both. Currently, data are insufficient to permit a definitive choice among these possibilities, nor are they necessarily mutually exclusive.

However, our results do not support hypothesis 1, because most of our patients with both PCT and HCV had acquired or sporadic PCT that apparently developed after their acquisition of HCV infection. Also, there is no known pathogenic basis for an increased risk of HCV infection in patients with PCT, unless poor techniques were used during therapeutic phlebotomies, leading to HCV infection by phlebotomists.

Hypothesis 2, that chronic HCV infection predisposes to development of PCT, seems more plausible. This is not attributable to a direct effect of HCV infection or chronic hepatitis on activity of hepatic Uro-D.<sup>40</sup> The pathogenesis of PCT is complex, not understood completely, and probably variable among different patients. The majority of subjects with the common familial form of PCT who have an inborn 50% decrease in Uro-D activity never develop overt PCT.<sup>1-3</sup> Thus, such a defect is neither necessary nor sufficient to ensure the development of overt clinical PCT. Usually, a decrease of 50% in activity of Uro-D is insufficient to limit uroporphyrinogen decarboxylation or overall heme synthesis, and increased production of uroporphyrinogen (due to induction of 5-aminolevulinic acid synthase) does not occur. (In contrast, all subjects with severe [homozygous or compound heterozygous] deficiency of Uro-D do develop severe clinical manifestations as part of the rare disease called hepatoerythropoietic porphyria.<sup>1-3</sup>) Overall, 18% of our PCT patients (30% of those HCV-positive and 14% of those HCV-negative) had evidence of an inherited partial ( $\approx 50\%$ ) defect in Uro-D (Table 2), suggesting that such a defect is not a significant predominant risk factor for PCT in conjunction with chronic HCV infection.

Therefore, other factors (e.g., alcohol, estrogens, iron) must play important roles in the pathogenesis of PCT. Alcohol has been thought to act by causing liver damage, and, as confirmed here, evidence of liver injury is known to occur in essentially all patients with overt PCT. Alcohol also exacerbates the hepatopathy of chronic HCV infection.<sup>41</sup> It is probable that many patients with PCT who formerly were thought to have alcohol-induced liver injury in fact had chronic hepatitis C or combined chronic HCV and alcohol-induced injury. Alcohol, iron, and chronic HCV infection all produce increased oxidative stress in liver cells,<sup>21-23,42</sup> perhaps increasing the likelihood that uroporphyrinogen in hepatocytes will oxidize to uroporphyrin, which is not a substrate for Uro-D and therefore will accumulate. In sufficient concentration, hepatocytic uroporphyrin precipitates as crystals,<sup>20</sup> most notably in proximity to storage iron.<sup>20</sup> In addition, other nonporphyrin products of uroporphyrinogen oxidation, the formation of which is stimulated by iron or haloaromatic compounds, can inhibit Uro-D and further increase accumulation of uroporphyrinogen and uroporphyrin.<sup>1-3,22</sup> Increased oxidative stress may help to produce deficiency of ascorbic acid, low plasma levels of which have been described in HCV-positive and HCV-negative patients with PCT.<sup>43</sup> In an experimental model of PCT, ascorbate abrogated uroporphyrin-

TABLE 6. Summary of Studies on PCT and Chronic Hepatitis C

1st Author, y	Ref.	City/Country of Origin	All Patients With PCT				(in patients with chronic hepatitis C and PCT)								Comments
			Total No.		No. (%)		Risk Factors for PCT No. (%)				Risk Factors for HCV No. (%)				
			Studied	Sex M/F	With HCV	HbS Ag	Alcohol	Familial	Other	None	IVDU	MSP	Other	None	
Fargion, 1992	6	Milan, Italy	74	70/4	56/74 (76%)	4/74 (5%)	28/74 (38%)	4/74 (5%)	1/74 (1%)	44/74 (55%)	0/74 (0%)	NR	0/74 (0%)	NR	Familial PCT by history only; 3/4 HCV+
Siersema, 1992	10	Rotterdam, Netherlands	38	25/13	7/38 (18%)	0/38 (0%)	27/38 (71%)	2/22 (9%)	2/38 (5%)	4/38 (11%)	NR	NR	NR	NR	Familial PCT defined by Uro-D
LaCour, 1993	5	Nice, France	13	12/1	10/13 (77%)	1/13 (8%)	8/13 (62%)	0/10 (0%)	2/10 (15%)	3/13 (23%)	1/10 (10%)	NR	3/10 (30%)	6/10 (60%)	All said to be "sporadic" PCT, but no Uro-D activities done. 4/13 had H/O blood trans- fusion.
Herrero, 1993	4	Barcelona, Spain	100	87/13	75/100 (75%)	NR	71/100 (71%)	5/100 (5%)	NR	NR	3/100 (3%)	NR	NR	NR	Familial PCT by history only. Prevalence of HCV positivity increased with histologic severity. Most HCV – pts normalized serum ALT after PCT therapy
Murphy, 1993	7	Dublin, Ireland	20	14/6	2/20 (10%)	0/20 (0%)	NR	NR	NR	NR	NR	NR	NR	NR	HCV rarely a risk factor for PCT in Ireland
De Castro, 1993	35	Madrid, Spain	62	56/6	24/34 (71%)	1/62 (2%)	24/34 (71%)	2/24 (8%)	NR	NR	1/24 (4%)	1/24 (4%)	7/24 (29%)	13/24 (54%)	Familial PCT by history only. Serum ALT and AST higher in HCV+ pts; other lab studies not different
Ferri, 1993	36	Florence and Pisa, Italy	23	23/0	21/23 (91%)	0/23 (0%)	6/21 (29%)	0/22 (0%)	NR	NR	0/21 (0%)	NR	2/21 (10%)	NR	Familial PCT by history only. 2/23 had H/O blood transfusion or "contact with hepa- titis viruses"
Koester, 1994	13	Albuquerque, NM	5	5/0	5/5 (100%)	0/5 (0%)	NR	NR	NR	NR	NR	NR	NR	NR	Small series from U.S.A.
Andreone, 1995	37	Bologna, Italy	9	9/0	8/9 (89%)	0/9 (0%)	NR	NR	NR	NR	NR	NR	NR	NR	6/9 HCV+ by EIA II and RIBA II
Cribier, 1995	28	Strasbourg, France	13	12/1	7/13 (54%)	0/13 (0%)	4/7 (57%)	0/7 (0%)	NR	NR	2/7 (28%)	NR	3/7 (43%)	2/7 (28%)	All negative for anti-HIV. One familial PCT by history and URO-D HCV genotypes: 1 in 4 pts; 2 in 2 pts; 1 + 2 in 1 pt.
Gibson, 1995	11	Melbourne, Australia	112	74/38	26/112 (23%)	NR	NR	NR	NR	NR	NR	NR	NR	NR	Retrospective study of stored sera, assayed only by EIA 2 test for antibodies to HCV
Lim, 1995	14	New York, NY	6	3/1	3/4 (75%)	0/4 (0%)	2/3 (67%)	0 (0%)	NR	NR	NR	NR	NR	NR	Only ¼ had abnormal liver chemistries
Stolzel, 1995	8	Marburg, Germany	106	66/40	8/106 (8%)	1/106 (1%)	2/8 (25%)	1/8 (13%)	0/8 (0%)	5/8 (60%)	NR	NR	NR	NR	Familial PCT by history only. Lab tests, including ALT and AST, not different in HCV+ v HCV – pts.
Salmon, 1996	12	Waikato, New Zealand	25	NR	0/25 (0%)	1/25 (4%)	← (No patients had hepatitis c) →								In New Zealand, HCV is not a risk factor for PCT, whereas alcohol and estrogen use are
Andant, 1997	39	Paris, France	124	NR	26/124 (21%)	NR	NR	41/124 (33%)	NR	NR	7/26 (27%)	NR	6/26 (23%)	NR	Familial PCT by RBC Uro-D
Bonkovsky, 1998	This paper	Several cities, USA	70	39/31	39/70 (56%)	1/39 (3%)	35/39 (90%)	7/39 (18%)	5/39 (13%)	0/39 (0%)	11/27 (41%)	13/27 (48%)	0 (0%)	12/27 (44%)	Familial PCT by RBC Uro-D. Liver tests not different in HCV+ vs. HCV – pts.
Totals			800	495/154	317/770 (41%)	9/431 (2%)	207/337 (61%)	62/391 (16%)	10/196 (6%)	56/172 (33%)	25/289 (8.7%)	14/51 (27%)	21/162 (13%)	33/68 (49%)	

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; EIA, enzyme-linked immunoassay; H/O, history of; IVDU, intravenous illicit drug use; MSP, many sex partners; NR, data not reported; pts., patients; RIBA, recombinant immunoblot assay.

rin accumulation.<sup>44</sup> Currently, it is unknown whether chronic HCV infection, with PCT, is associated with hepatic ascorbic acid deficiency.

With respect to hypothesis 3, namely, that others factors predispose to both PCT and chronic HCV, the most appealing candidates are alcohol and iron. The influences of alcohol abuse and hepatic iron overload on PCT are well known.<sup>1-3</sup> Recent results from Northern Europe have shown a strikingly high prevalence (44%-47%) of the C282Y mutation of the HFE gene in patients with PCT, with about one half of these being homozygous.<sup>15,16</sup> In contrast, among 68 male PCT patients from northern Italy, the prevalence of the C282Y mutation was low (3%) and not different from controls. However, in the Italian men, there was an increased prevalence (50%) of a second mutation (H63D) described in the HFE gene, with 15% being homozygous for this mutation.<sup>45</sup> Our results for North American patients with PCT show increased prevalences of both mutations (42% and 31%, respectively) and of homozygosity (36% for the C282Y and 25% for the H63D mutation). In our patients, these two mutations never occurred together in the same patient. Thus, overall, 73% of our patients had defined mutations in the HFE gene, strongly supporting a key role of such mutations

in the pathogenesis of PCT, through effects on iron metabolism.

An important role of the C282Y mutation in iron overload is supported by several studies.<sup>17,46</sup> In contrast, a role for the H63D mutation in causing iron overload has been less clear. However, it does appear that the latter mutation, especially in compound heterozygotes (C282Y +/–, H63D –/+ ) is associated with iron overload, albeit less than that associated with homozygosity for C282Y and/or the ancestral haplotype of HLA-linked hereditary hemochromatosis.

The importance of iron in the pathogenesis of PCT is well established. Iron depletion remains the cornerstone of therapy for PCT, regardless of HCV status.<sup>1-3</sup> Iron depletion also ameliorates the severity of chronic hepatitis C<sup>21,23</sup> and may improve its response to interferon.<sup>21-23,48</sup> In contrast, patients with iron overload (e.g., HLA-linked hereditary hemochromatosis) are at increased risk for development of both PCT and HCV infection.<sup>48</sup> Similarly, chronic, heavy alcohol use may exacerbate HCV infection, increase the likelihood of development of chronic hepatitis, and diminish its responsiveness to interferon.<sup>41</sup>

Despite the association of chronic hepatitis and PCT, most



patients with chronic HCV infection do not develop PCT. In our survey of 110 patients with chronic hepatitis C, fewer than 5% had a PCT-like abnormality in the pattern of urinary porphyrin excretion (i.e., increased uro- and heptacarboxyporphyrins [Fig. 1]). More frequent was the coproporphyrinuria, typical of many liver and other diseases.<sup>1,2,40,49</sup> Thus, routine porphyrin testing of all HCV-infected patients is not indicated, but should be limited to those with features suggestive of PCT (or other types of porphyria). The reasons for the differences in urinary porphyrin profiles in PCT patients with or without HCV infection (Fig. 1) are unknown, but may reflect a differential effect on the several decarboxylation steps catalyzed by Uro-D. Careful study of additional PCT patients with and without HCV infection is needed to confirm our findings.

In Italy, a highly significant association between infection with HCV of genotype 1b and risk of development of PCT has been described,<sup>50</sup> and 7 of 8 PCT patients from Chile who had HCV RNA in the blood also carried genotype 1b.<sup>51</sup> In contrast, 6 of our 7 patients who had chronic HCV and PCT whose HCV could be genotyped were found to carry viruses of genotype 1a. Genotype 1 HCV tends to cause severe and progressive chronic hepatitis, but it appears that either genotype 1a or 1b may be associated with development of PCT. French patients with PCT and HCV infection with non-type 1 genotypes have been described,<sup>39</sup> as has a Japanese man with PCT complicating chronic alcohol abuse and HCV infection caused by a genotype 2 strain.<sup>52</sup> In the latter patient, both the PCT and the HCV infection responded to interferon therapy. Thus, infection with HCV of genotype 1 is not a *sine qua non* for development of PCT complicating chronic hepatitis C.

An association of PCT and infection with the human immunodeficiency virus (HIV) has been suggested.<sup>53-55</sup> However, most patients with both PCT and HIV infections have other risk factors for development of PCT, including heavy alcohol use, iron overload, and/or concomitant HCV infection.<sup>55</sup> Thus, whether HIV is in fact an independent risk factor for PCT is contentious. Because of the stringent rules to assure confidentiality of HIV test results currently in force in the United States, we were not able to obtain such results on our patients.

In summary, chronic HCV infection (56%) and mutations in the HFE gene (73%) are highly prevalent in North American patients with PCT. Most clinical and laboratory features of PCT patients do not predict which patients will be positive for chronic hepatitis C, and all should have serological tests for antibodies to HCV, and mutational analysis of the HFE gene, particularly if they have relatives who may be at risk for hemochromatosis. Initial management of overt PCT should continue to be the removal of offending chemicals (alcohol, estrogens, haloaromatic compounds, iron) and iron depletion by vigorous phlebotomy. Those with chronic hepatitis C may derive additional benefit from such iron-reduction therapy, in amelioration of hepatic inflammation, and responsiveness to interferon therapy.<sup>21-23</sup>

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