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Porphyria Cutanea Tarda in Brazilian Patients: Association With Hemochromatosis C282Y Mutation and Hepatitis C Virus Infection

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OBJECTIVE: Porphyria cutanea tarda (PCT) is commonly associated with iron overload and hepatitis C virus (HCV) infection. Association between hemochromatosis C282Y or H63D mutations and PCT has been observed, although not uniformly, and iron overload is also commonly found in chronic HCV hepatitis. The aim of the present study was to investigate the frequency of C282Y and H63D mutations and HCV infection in Brazilian patients with PCT and their relationship with iron overload.

METHODS: Twenty-three patients (19 men) aged 39.6 ± 11.1 yr were studied. All had dermatological lesions of PCT and high levels of urinary uroporphyrin. HCV infection and iron overload were investigated. DNA samples were analyzed for the presence of HFE mutations.

RESULTS: The frequency of C282Y was significantly higher in PCT patients than in 278 healthy individuals (17.4% *vs* 4%, odds ratio = 5.1, 95% confidence interval 1.5–17.6, $p = 0.02$), whereas no difference was observed regarding the H63D mutation (30.4% *vs* 31%, odds ratio = 1, 95% confidence interval 0.4–2.4, $p = 1$). Biochemical tests in PCT patients showed iron overload with transferrin saturation = 47.3 ± 20.7% and ferritin = 566.8 ± 425 ng/ml. Fifteen of 23 (65.2%) patients had HCV infection and alcohol ingestion was observed in 17 of 23 (73.9%).

CONCLUSIONS: PCT patients exhibited evidence of iron overload, a high frequency of HCV, and an association with C282Y mutation. These data further support the notion that both acquired and inherited factors contribute to the occurrence of PCT, and indicate that screening for C282Y may be justified in PCT patients. (Am J Gastroenterol 2000;95: 3516–3521. © 2000 by Am. Coll. of Gastroenterology)

INTRODUCTION

Porphyria cutanea tarda (PCT) is the most common form of porphyria, a group of disorders characterized by deficiency of enzymes of the heme biosynthetic pathway that results in accumulation of precursor substrates. Most cases of PCT are sporadic, resulting from an acquired defect involving reduced activity of uroporphyrinogen decarboxylase (1). PCT is clinically characterized by skin lesions on sun-exposed areas, liver abnormalities, and high levels of porphyrins in plasma and urine (2). The liver abnormalities include steatosis, portal fibrosis, cirrhosis, siderosis, and hepatocarcinoma (3). Alcohol abuse, drugs, exposure to agricultural pesticides, iron overload, and viral infections can be triggering factors that precipitate a latent enzymatic defect and consequently the clinical manifestation of PCT (2, 4).

Several studies have shown an association between PCT (sporadic type) and hepatitis C virus infection (5–12). In some areas this association is very strong (5–10), similar to our experience (13).

Iron overload has been frequently observed in patients with PCT (2–4) and in patients with chronic hepatitis C virus (HCV) infection (14) as well. However, the exact reason why increased iron accumulation occurs in these patients is still unclear.

Recently, two mutations in the HFE gene on chromosome 6 were discovered to be associated with hereditary hemochromatosis (HH), which is the most common genetic disease affecting European whites (15–17). The 845 G→A (C282Y) mutation is more clearly associated with HH being indeed currently considered a disease-causing mutation (15, 16). The relationship of the second mutation (187 C→G; H63D) with HH is less evident but its presence appears to result in hemochromatosis in some patients when coinherited with the C282Y mutation (15, 16, 18–21). The description of these mutations prompted us to test their association with PCT, a disease in which body iron accumulation is well known to exist.

In this study we investigated the frequency of C282Y and H63D mutations and HCV infection in patients with PCT and its relationship with iron overload.

MATERIALS AND METHODS

Thirty-six patients with a clinical and laboratory diagnosis of PCT were seen at our hospital (University Hospital, School of Medicine of Ribeirão Preto, University of São Paulo) from January 1982 to December 1998, and received a letter inviting them to participate in this study. Twenty-three patients (63.8%) agreed to participate and were eventually enrolled. The study group comprised 19 men and 4 women aged 22 to 64 yr (mean [± SD] = 39.6 ± 11.1 yr). All patients had dermatological lesions of PCT and high levels of urinary uroporphyrin (mean = 2185 ± 1961 μg/24 h; normal range = 0–25 μg/24 h) and coproporphyrin (mean = 558 ± 482 μg/24 h; normal range = 20–200 μg/24 h).

Patients were submitted to clinical evaluation and risk factors for viral infection (blood transfusion, tattoo, and drug abuse), history of alcohol abuse, and chronic use of medicines were investigated. Informed consent was obtained from all individuals participating in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local Ethics Committee.

Blood samples were collected and sera were tested for HCV infection (second generation IgG antibody to recombinant HCV antigens, Abbott Laboratories, , and HCV RNA, Amplicor HCV test, Roche Diagnostic Systems, NJ), HBV infection (HbsAg by ELISA; antibodies to HbsAg by EIA, and antibodies to HBcAg by EIA; all HBV tests from Abbott Laboratories), and for antibodies to HIV (anti-HIV; ELISA, Abbott Laboratories). HCV genotyping was performed by INNO-LiPA HCV (Innogenetics, Belgium).

Blood samples were also tested for liver enzymes (AST, ALT, and gamma-glutamyltranspeptidase [GGT]), iron, transferrin saturation, and ferritin. HCV genotyping was performed in 10 patients.

A liver biopsy was available for analysis in 11 patients, and was performed after routine criteria for diagnosis or clinical management of liver disease. Specimens were fixed in 10% formol–saline and processed by the usual paraffin technique. Routine stains included hematoxylin and eosin and staining for connective tissue and iron (Perl's stain). Slides of 10 biopsies were scored for necroinflammatory activity (HAI) and stage using the score system of Knodell *et al.* (22), modified by Desmet *et al.* (23). Iron deposits were assessed in 11 biopsies and scored on the basis of both amount and cellular and lobular location using the score system of Brissot *et al.* (24), modified by Sciot *et al.* (25).

Genomic DNA was extracted from peripheral blood leukocytes by standard methods. DNA analysis was performed by polymerase chain reaction amplification followed by restriction-enzyme digestion with *Rsa*I (for C282Y mutation analysis) and *Bcl*I (for H63D mutation analysis). Primers and polymerase chain reaction conditions were previously described (17). DNA samples from all patients with PCT were analyzed for the presence of both mutations. In addition, the same analysis was performed for DNA samples from 278 healthy subjects (blood donors and hospital medical staff) from the same geographical area and have the same ethnic background.

Statistical Analysis

Data were analyzed by nonparametric tests (Mann-Whitney and Fisher's tests). The statistics were two-tailed. $p < 0.05$ was considered significant. Values are given as mean ± 1 SD. Odds ratio (OR) as a measure of relative risk was calculated in the standard unmatched fashion using the Instat 3.00 package (Graphpad Software, San Diego, CA). A 95% confidence interval (95% CI) was calculated according to standard techniques.

RESULTS

Clinical Characteristics

Clinical details of the 23 PCT patients are presented in Tables 1 and 2. A high predominance of men was observed. History of alcohol ingestion was found in 17 of 23 (73.9%) patients. The mean daily ethanol consumption was >60 g in 15 patients and about 40 g in the other two. Three of 23 patients had a history of chronic ingestion of oral contraceptives, one was taking an antiepileptic drug (phenytoin) and one had a history of exposure to agricultural pesticides.

HFE Mutations

Gene HFE mutations were observed in 10 of 23 (43.5%) PCT patients. Four of 23 (17.4%) carried C282Y and 7 of 23 (30.4%) carried H63D. One patient had compound mutation. H63D mutation was detected in the homozygous state in one patient and in the heterozygous state in six patients. No homozygous for C282Y mutation was found.

When the frequencies of mutations observed in PCT patients (C282Y: 17.4%; H63D: 30.4%; any of the two mutations: 43.5%) were compared with those for healthy individuals (C282Y: 4%; H63D: 31.1%; any of the two mutations: 34.9%) from the same geographic area there was a statistically significant difference for C282Y mutation (OR = 5.1, 95% CI 1.5–17.6, $p = 0.02$). No difference was observed for H63D (OR = 1, 95% CI 0.4–2.4, $p = 1$).

Biochemical Parameters

The individual results of laboratory tests are shown in Table 1. Biochemical tests in the 23 PCT patients revealed iron overload and elevated serum liver enzymes (results for the whole group are showed in Table 2). No significant difference ($p > 0.05$) was observed between patients carrying or not carrying HFE mutations regarding any of the biochemical parameters tested (iron = 144 ± 73 μg/dl *vs* 138 ± 61 μg/dl; transferrin saturation = 51 ± 23% *vs* 49 ± 22%; ferritin = 681.6 ± 521 ng/ml *vs* 516 ± 296 ng/ml; AST = 65 ± 45 U/L *vs* 73.4 ± 45 U/L; ALT = 69 ± 46 U/L *vs* 93 ± 59 U/L; GGT = 99 ± 71I U/L *vs* 138 ± 105 U/L, respectively).

Table 1. Individual Clinical and Laboratory Characteristics of 23 PCT Patients

Subject	Date, PCT Diagnosis	Age/Sex	Alcohol Ingestion	URO (μg/24 h)	COPRO (μg/24 h)	HCV/ Genotype	HIV	GGT (U/L)	AST (U/L)	ALT (U/L)	Iron (μg/dl)	Transf. sat. (%)	Ferritin (ng/ml)	HFE Mutation
1	1997	54 M	Yes	124	62	p/1b	n	286	90	116	202	86	364	No mutation
2	1996	32 M	Yes	2960	470	p/1a	n	217	56	80	83	32	462	No mutation
3	1986	52 M	Yes	338	205	p/3a	n	39	19	38	77	32	788	No mutation
4*	1991	46 M	Yes	1700	477	p/1a	n	48	79	112	158	58	621	No mutation
5	1998	26 M	Yes	3418	1026	p	p	219	103	233	171	57	337	No mutation
6	1998	44 M	No	1454	41	p	n	200	70	82	125	39	NA	No mutation
7	1993	26 M	Yes	193	210	p/1	p	114	157	86	93	25	322	No mutation
8	1997	47 M	Yes	8350	1008	n	n	90	105	143	147	63	886	No mutation
9	1996	39 M	Yes	905	337	n	n	35	18	26	NA	NA	495	No mutation
10*	1990	37 M	Yes	3405	1043	n	n	17	17	16	83	29	177	No mutation
11	1998	54 M	Yes	3530	2115	n	n	321	59	53	171	55	1108	No mutation
12	1998	36 M	Yes	887	576	p	n	186	139	148	274	57	541	No mutation
13*	1997	28 F†	No	1869	211	n	n	25	42	72	72	25	90	No mutation
14	1994	44 M	Yes	330	194	p/1a	n	75	132	146	173	78	446	H63D HOM
15	1996	40 M	Yes	3508	994	p/3a	p	99	123	133	71	17	764	H63D HET
16*	1982	64 F‡	No	100	103	p/1b	n	86	20	24	104	53	142	H63D HET
17	1992	33 M	Yes	3126	397	p/1b	p	123	70	57	210	58	628	H63D HET
18	1998	27 M	Yes	1889	576	p	p	254	67	74	136	50	650	H63D HET
19*	1992	32 F†	No	4648	672	n	n	12	34	45	61	21	233	H63D HET
20	1989	47 M	Yes	3894	876	p/1	n	62	119	114	293	89	1123	C282Y HET
21*	1997	22 F†	No	557	119	n	n	26	38	46	113	39	322	C282Y HET
22*	1990	31 M§	Yes	2614	564	n	n	37	17	37	139	51	1817	C282Y HET
23	1998	51 M	No	581	19	p	n	144	30	19	NA	NA	NA	H63D/C282Y

* PCT had been in remission when the iron or liver function tests were performed. History of use of oral contraceptives (†), phenytoin (‡), and exposure to agricultural pesticides (§) are indicated.

NA = results before phlebotomy not available; HOM = homozygous; HET = heterozygous; p = positive; n = negative; M = male; F = female.

HCV Infection and Other Virus

Fifteen of 23 PCT patients (65.2%) were anti-HCV positive. All of them had HCV–RNA-positive serum. Five of 15 were anti-HIV positive, 5 of 15 were anti-HBc positive, and 2 of 15 were both anti-HBc and anti-HIV positive. Ten of 15 HCV patients had the genotype of HCV determined, which revealed type 1 in 8 (3 subtype 1a; 3 subtype 1b, and 2 not subtyped), and subtype 3a in 2 patients.

The remaining eight PCT patients were anti-HCV/HCV–RNA negative; all of them were also anti-HIV negative. HBsAg was not detected in any of the 23 PCT patients.

Comparing HCV-positive with HCV-negative patients we observed significantly higher levels in the former regarding GGT (143 ± 79 U/L *vs* 70 ± 104 U/L, $p = 0.008$), AST (85 ± 43 U/L *vs* 41 ± 30 U/L; $p = 0.01$), and ALT (97 ± 56 U/L *vs* 55 ± 39 U/L, $p = 0.04$). No difference was observed between these two groups regarding serum ferritin levels (490 ± 309 ng/ml *vs* 720 ± 595 ng/ml, $p = 0.29$), transferrin saturation (52 ± 22% *vs* 38 ± 16%, $p = 0.1$), or serum iron levels (155 ± 71 μg/dl *vs* 106 ± 42 μg/dl, $p = 0.08$).

Table 2. Clinical and Laboratory Characteristics of 23 PCT Patients

Characteristics	PCT patients (N = 23)
Sex (male:female)	19:4
Age (yr)	39.6 ± 11.1
C282Y mutation	4/23 (17.4%)
H63D mutation	7/23 (30.4%)
Alcohol ingestion	17/23 (73.9%)
Serum iron (μg/dl)	153 ± 65 (men)
	87.5 ± 25 (women)
Transferrin saturation (%)	53 ± 22 (men)
	35 ± 14 (women)
Ferritin (ng/ml)	678 ± 395 (men)
	197 ± 102 (women)
Serum HCV–RNA positive	15/23 (65.2%)

Normal range: iron = 49–151 μg/dl (women) and 53–167 μg/dl (men); ferritin = 18–370 ng/ml (men) and 9–120 ng/ml (women).

HCV infection was observed in 7 of 10 patients carrying an HFE mutation and in 8 of 13 noncarriers. One of seven patients was homozygous for H63D, four were heterozygous for H63D, one heterozygous for C282Y, and one had compound mutation.

Twelve of 15 HCV patients had a history of alcohol ingestion. Three had received blood transfusion in the past and six had a history of intravenous drug addiction. Tattoos were not observed.

Liver Biopsy

Liver biopsy performed in 11 of 23 patients showed chronic hepatitis in all of them. All except one had HCV infection. Four had HFE mutation (one homozygous for H63D, two heterozygous for H63D, and one heterozygous for C282Y). Iron deposits in liver were observed in four of four patients carrying HFE mutation and in four of seven not carrying HFE mutation. Scores of iron deposits did not differ between carriers (7.7 ± 5) and noncarriers (4 ± 5.6). The scores of necroinflammatory activity (HAI) also did not

Table 3. Studies Evaluating the Frequency of HFE Gene Mutations in PCT Patients

Reference	Geographic Origin	Number of Patients	C282Y (PCT *vs* Controls)	OR (95% CI) (C282Y)	H63D (PCT *vs* Controls)	OR (95% CI) (H63D)
Roberts *et al.* (26)	UK	41	44% *vs* 11%	6.4 (2.6–15.4)	24.4% *vs* 24.8%	1 (0.4–2.3)
Stuart *et al.* (27)	Australia	27	44.4% *vs* 12%	6.5 (2.4–17.5)	44.4% *vs* 24.8%	2.4 (1–5.9)
Bonkovsky *et al.* (28)*	USA	26	42% *vs* 14%	4.5 (1.8–10.8)	31% *vs* 26.9%	1.2 (0.5–2.9)
Sampietro *et al.* (29)	Italy	68	2.9% *vs* 1.5%	1.9 (0.3–13.8)	50% *vs* 24%	3.1 (1.6–5.8)
Bulaj *et al.* (30)	USA	87	41.4% *vs* 12.5%	4.9† (2–12.1)	28.7% *vs* 19.6%	1.6 (0.7–3.7)
Martinelli *et al.* (present study)	Brazil	23	17.4% *vs* 4%	5.1 (1.5–17.6)	30.4% *vs* 31.1%	1 (0.4–2.4)

* To calculate the OR in this study we considered as data from control populations those shown by Burke *et al.* (36).
† OR calculated by taking into account all C282Y carriers; OR for C282Y homozygous is 60 (95% CI 18.5–195.7).

differ between patients carrying (9.7 ± 3.4) or not carrying (9.3 ± 2.6) HFE mutations.

DISCUSSION

In the present investigation we observed a significantly higher frequency of the C282Y HFE mutation in patients with PCT than in healthy controls. This finding suggests that carriership of C282Y results in an increased risk for the occurrence of PCT.

To the best of our knowledge, this is the first investigation dealing with the relationship between HH and PCT in South America. Our data indicate that C282Y is a genetic factor associated with the occurrence of PCT in the Brazilian population, as possession of the mutant allele increased the risk of PCT 5-fold. To better evaluate the risk of HH for PCT, we analyzed the results from five other studies (26–31) in the literature in which this issue was investigated, and these data are shown in Table 2. Association between PCT and C282Y mutation was observed in British and Australian patients (24, 25). The investigators found C282Y mutation in 44% and 44.4% of PCT patients and in 11% and 12% of the controls in UK and Australia, respectively. The frequency of H63D mutation did not differ between controls and PCT patients in either study. Bonkovsky *et al.* (28) observed in North American PCT patients frequencies of 42% and 31% of C282Y and H63D mutation, respectively. As shown in Table 2, the frequency of C282Y (but not of H63D) was higher in PCT than in a control population. In contrast, Sampietro *et al.* (29) did not find an association between C282Y and PCT in Italian patients, but a significantly higher prevalence of the H63D (50%) was observed in these patients compared with controls (24%). Recent data by Bulaj *et al.* (30) point to a major role of the homozygous state for C282Y in determining the risk of PCT (OR: 60; 95% CI 18.5–195.7). In Table 3, we calculated the OR for PCT in the article by Bulaj *et al.* by grouping all C282Y carriers together, and found an OR of 4.9 (95% CI 2–12.1). It should be mentioned, however, that C282Y heterozygosity was not overrepresented in PCT patients in the study by Bulaj *et al.* Taken together, the data from these studies, except one (29), point to a significant role of C282Y in determining the risk of PCT. It should be noted that the recently reported polymorphism in intron 4 of the HFE gene (31) did not affect our findings, as a mutant C282Y homozygote was not found in the present investigation.

In the present study a high prevalence of HCV infection was found in PCT patients, as also observed by other researchers (5–10). Although this association has been frequently observed, it is not a rule (12). In our study a high percentage of patients with the gene HFE mutation also had HCV infection; however, patients with HCV infection did not show a higher risk of carrying HFE mutations. In a previous study we did not find a difference in the frequency of HFE mutations comparing patients with chronic HCV infection and healthy controls from the same geographical area (32). However, we observed that HCV patients carrying HFE mutations had more severe liver disease (32). We also demonstrated previously that HCV patients may exhibit abnormalities in uroporphyrin metabolism even without clinical manifestations of PCT (33).

It should be noted that, although we could find a significant association between C282Y and the occurrence of PCT, carriership of this mutation does not explain by itself iron overload in most patients with PCT or HCV infection in our study. In fact, only 13% of the PCT patients carried the C282Y mutation. This finding suggests the presence of other still unidentified factors contributing to the iron accumulation process usually found in PCT.

Genotype 1a, 1b, and 3a were detected in our patients with HCV infection in accordance with other investigators showing that genotype 1 infection, although the most common, is not the only one associated with PCT (34).

The observation that PCT patients with HCV infection had significantly higher levels of serum liver enzymes was expected because they have an additional factor for liver injury. Biochemical parameters of iron overload did not differ between patients with and without HCV infection. HCV infection is associated with iron overload even in patients without PCT (14). We could not show that the association of factors enhanced iron overload. However, the small number of patients limited these analyses. In addition, the percentage of alcohol ingestion was very high in our study population as 73.9% of HCV patients consumed alcohol. Alcohol is considered an important triggering factor

for PCT (2, 4) and alcoholic liver disease is also associated with iron overload (35).

Iron overload was common in PCT patients either with or without C282Y or H63D mutations. The presence of HFE mutations was not associated with differences in the values of serum or liver parameters of iron overload, scores of activity of liver disease, or of iron deposits in the liver. Thus, considering these data as a whole, we could not find evidence that the presence of HFE mutations implies either greater activity of liver disease or greater iron accumulation. We should point out, however, that the small number of liver biopsies as well as the small number of HFE carriers identified limited these analyses. Moreover, the coexistence of factors causing liver injury and iron overload such as alcohol ingestion or HCV infection was very frequently observed and may have been a confounding factor.

In conclusion, C282Y carriership was found to increase the risk of PCT in the Brazilian population, which supports current literature. Furthermore, HCV infection, body iron overload, and alcohol ingestion were common in PCT patients. Taken together, these data emphasize the notion that both genetic and acquired factors play a role in determining the risk of PCT, which, in this sense, may be seen as a multifactorial disease. Finally, our data suggest that screening for C282Y may be justified in patients with an established diagnosis of PCT.

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