

High Prevalence of the His63Asp HFE Mutation in Italian Patients With Porphyrria Cutanea Tarda

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Sporadic porphyria cutanea tarda (PCT) is caused by a reduced activity of uroporphyrinogen decarboxylase (URO-D) in the liver. Mild to moderate iron overload is common in PCT, as iron is one of the factors which trigger the clinical manifestations of the disease through the inactivation of URO-D. A role for genetic hemochromatosis in the development of iron overload in sporadic PCT has been hypothesized in the past. The aim of this work was to investigate whether mutations of HFE, which is a candidate gene for hemochromatosis, play the role of genetic susceptibility factors for PCT in Italian patients, who have a high prevalence of acquired triggering factors, such as hepatitis C virus (HCV) chronic infection and alcohol. We determined HFE genotypes of 68 male patients with PCT. Our data do not confirm an association of PCT with the Cys282Tyr HFE mutation, strongly associated with hemochromatosis in Northern European countries. A second mutation of HFE, His63Asp, however, had a significantly increased frequency as it was present in half of the patients. Surprisingly, the presence of the His63Asp mutation was not related to the iron status of patients, suggesting that a subtle abnormality of iron metabolism induced by this mutation could escape detection by the standard parameters of iron status. In PCT patients with liver disease, the presence of the mutation could contribute to the inactivation of URO-D, either directly or through a synergistic action with other factors that cause liver damage. (HEPATOLOGY 1998;27;181-184.)

Sporadic porphyria cutanea tarda (PCT), the most common type of porphyria,¹ is caused by a reduced activity of

uroporphyrinogen decarboxylase (URO-D) in the liver.² Liver disease is almost always present in PCT³ and mild to moderate iron overload is common. Iron is one of the factors that trigger the clinical manifestations of the disease,⁴⁻⁶ contributing to the inactivation of URO-D.⁷ Iron depletion is usually followed by the regression of cutaneous lesions,⁸ even in patients without evident iron overload, possibly caused by the presence of intra-hepatocytic toxic species of iron.

A possible role for the hemochromatosis gene in the development of iron overload in sporadic PCT has been hypothesized in the past⁹ and is indirectly supported by studies of human leukocyte antigen (HLA)-A3 allele frequency in groups of PCT patients with iron overload.¹⁰⁻¹³

A candidate gene for hemochromatosis encoding an HLA class I-like molecule, HFE, was recently identified.¹⁴ A missense mutation of HFE (Cys282Tyr) was reported as tightly associated with the classical hemochromatosis phenotype, which was present in the homozygous state in the large majority of patients of Northern European descent.¹⁴⁻¹⁷ A second mutation (His63Asp) was also found to have an increased frequency on hemochromatosis chromosomes that did not present with the Cys282Tyr mutation, but its relationship with hemochromatosis has not been clearly established.^{14,15}

The availability of a genetic marker for hemochromatosis allows for the direct investigation of its relationship with PCT. Roberts et al.¹⁸ reported a significant increase in the frequency of the Cys282Tyr mutation in British PCT patients, thereby confirming that inheritance of a mutation which causes hemochromatosis is an important factor in determining genetic predisposition to sporadic PCT.

Two major differences between the British and the Italian population make it interesting to establish the contribution of HFE mutations in Italian PCT patients. Hemochromatosis seems to be less genetically homogeneous in Southern Europe. The Cys282Tyr mutation was present in the homozygous state in about 90% of British patients with overt hemochromatosis but in less than 70% of Italian¹⁹ and of Southern French patients.²⁰ The distribution of factors that trigger PCT also shows some relevant geographical differences. In Italy and in other Mediterranean countries hepatitis C virus (HCV) infection is the single most frequent cause of liver disease in PCT patients,^{6,21,22} while it is rare in Northern European countries.^{23,24} The aim of this work is to investigate whether HFE mutations have a role as genetic susceptibility factors for PCT in a population with a high prevalence of an acquired triggering factor, such as HCV chronic infection.

Abbreviations: PCT, porphyria cutanea tarda; URO-D, uroporphyrinogen decarboxylase; HLA, human leukocyte antigen; HCV, hepatitis C virus.

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PATIENTS AND METHODS

Patients

We studied 68 male patients with sporadic PCT (range, 28-80 years; median 61). PCT was diagnosed on the basis of typical clinical features and urinary porphyrin excretion (urinary porphyrins range, 900-8,000 mg per 24 hours; mean \pm SD 3,700 \pm 1,800). None of the patients had a clinical picture or a family history of hemochromatosis. Patients with clinical manifestations of PCT underwent phlebotomy with removal of 300 mL of blood (equivalent to 150 mg of iron) every week, even in the absence of evident iron overload. Iron depletion was defined as transferrin saturation of < 20% and ferritin < 30 mg/L in the presence of mild anemia (hemoglobin < 11 g/dL in women and 12 g/dL in men).

The iron status at the time of diagnosis was categorized in three classes, as previously described.¹³ In brief, iron overload was diagnosed in patients in the presence of a transferrin saturation > 45%, iron removed to reach iron depletion > 2 g or by liver iron concentration, available in 21 patients, > 28 μ mol/g of dry tissue. Patients in whom the transferrin saturation was \leq 45%, in whom the iron removed was \leq 2 g, and in whom the liver iron concentration was \leq 28 μ mol/g were included in class 0. Patients in whom the transferrin saturation was > 45%, in whom iron removed was > 2 g, or in whom the liver iron concentration was > 28 μ mol/g were included in class I, or in class II if transferrin saturation was > 62% and the iron removed was > 4 g.

Markers of hepatitis B virus (HBV) and HCV infection were obtained in all patients. Alcohol abuse was defined by present or past alcohol intake > 80 g/day for more than 5 years. All diagnostic parameters, with the exception of iron removed, were obtained before any therapeutic intervention (phlebotomy or α -interferon). No patient had known environmental exposures to hepatotoxic or porphyrogenic chemicals or toxins.

A control group of 128 subjects without any evidence of liver disease or porphyria was formed by enrolling volunteer individuals among hospital staff and medical students (group A). The frequency of HFE mutations was also established in 50 patients who were infected with HCV, who showed no evidence of porphyria or hemochromatosis, and who had a histological diagnosis of chronic active hepatitis (group B).

Methods

After conversion to their methyl esters, urinary porphyrins were fractionated by high performance liquid chromatography.²⁵ Serum iron and total iron binding capacity were determined by standard methods. Liver iron concentration was performed according to Barry's method.²⁶ The presence of hepatitis C infection was investigated by serology (EIAIII Ortho Diagnostic Systems, Raritan, NJ) and HCV-RNA was detected as previously described.²⁷ A present or past HBV infection was investigated by hepatitis B surface antigen and by the antibody to hepatitis B core antigen (Abbott Laboratories, North Chicago, IL).

Genomic DNA was extracted from peripheral leukocytes by standard procedures or from serum samples as described previously.²⁸ The two mutations of HFE were detected after amplification by polymerase chain reaction (PCR)¹⁴ and restriction with *RsaI* for Cys282Tyr and *BclI/MboI* for His63Asp. Polymorphic alleles of the D6S265 and D6S105 microsatellites were analyzed by polyacrylamide gel electrophoresis after polymerase chain reaction amplification with specific primers, as previously described.^{29,30} The two microsatellite alleles D6S265-1 and D6S105-8 are in strong linkage disequilibrium with the hemochromatosis gene and are part of the ancestral haplotype. Allele D6S265-1 is present only on chromosomes carrying HLA-A3.^{29,31} The ancestral haplotype was not reported in a well chosen normal control population in Italy,²⁹ indicating that it is strictly associated with the hemochromatosis gene.

Statistical comparison of data by the Fisher's Exact test or by χ^2

TABLE 1. Allelic and Genotype Frequencies of Two Mutations of HFE in 68 Patients With PCT, in 128 Control Subjects From the General Population (Group A) and in 50 Patients With HCV Chronic Hepatitis and No Evidence of Porphyria (Group B)

	PCT (n = 68)	Controls		P
		Group A (n = 128)	Group B (n = 50)	
Alleles				
Cys282Tyr	2/136 (1.5%)	2/256 (0.7%)	2/100 (2.0%)	NS
His63Asp	39/136 (28.7%)	33/256 (12.9%)	12/100 (12.0%)	<0.001*
Genotypes				
Cys282/Tyr282	2/68 (2.9%)	2/128 (1.5%)	2/50 (4.0%)	NS
Tyr282/Tyr282	0/68	0/128	0/50	NS
His63/Asp63	29/68 (42.6%)	29/128 (22.6%)	8/50 (16.0%)	0.005*
Asp63/Asp63	5/68 (7.3%)	2/128 (1.5%)	2/50 (4.0%)	0.050*

Abbreviation: NS, not significant.

*PCT patients vs. Group A controls.

was performed by the statistical package Instat 2.01 (Graphpad Software, San Diego, CA). All statistics were two-tailed.

RESULTS

The frequencies of HFE mutations and genotypes of patients and controls are reported in Table 1. The frequency of Cys282Tyr mutation was not significantly different in PCT patients and controls. The two patients carrying Cys282Tyr were heterozygous for the mutation. The His63Asp mutation was found on 28.7% of chromosomes from patients with PCT, a frequency significantly increased as compared with 12.9% of controls from the general population (group A) ($P = .0002$). The frequency of the same mutation in controls with HCV chronic hepatitis (group B) was not increased as compared with the general population. Thirty-four of 68 PCT patients (50%) carried His63Asp in the heterozygous or homozygous state versus 31 of 128 (24.1%) control individuals ($P = .0004$). One patient was a compound heterozygote for the two mutations.

On the basis of criteria chosen to define the iron status, 16 patients were classified as having a normal iron status (class 0); 39 patients were included in iron overload class I; and 13 patients were included in iron overload class II. The allelic and genotype frequencies of HFE mutations in patients belonging to the three classes of iron status are reported in Table 2. The Cys282Tyr mutation was reported in only two patients with iron overload, one in class I and one in class II.

TABLE 2. Allelic and Genotype Frequencies of Two Mutations of HFE in 68 Patients With PCT Sorted According to Their Iron Status

	Class 0 (n = 16)	Class I (n = 39)	Class II (n = 13)	P
Alleles				
Cys282Tyr	0/32	1/78 (3.3%)	1/26 (3.8%)	NS
His63Asp	12/32 (37.5%)	23/78 (29.5%)	6/26 (23.1%)	NS
Genotypes				
Cys282/Tyr282	0/16	1/39 (2.6%)	1/13 (7.7%)	NS
Tyr282/Tyr282	0/16	0/39	0/13	NS
His63/Asp63	6/16 (37.5%)	17/39 (43.6%)	6/13 (46.1%)	NS
Asp63/Asp63	3/16 (18.7%)	3/39 (7.7%)	0/13	NS

NOTE. Iron class status as follows: class 0, normal iron status; class I, mild to moderate iron overload; and class II, severe iron overload.

Abbreviation: NS, not significant.

The distribution of the His63Asp mutation was not significantly different in the three classes of iron status.

The ancestral hemochromatosis haplotype was identified in five PCT patients carrying D6S265-1 and D6S105-8 alleles, because of homozygosity for one or both alleles or through a pedigree analysis; in two patients, also heterozygous for both alleles, we were unable to assign haplotypes. All the patients having the ancestral haplotype had iron overload: two were in class I and three in class II. One of the patients in class II was homozygous for the ancestral haplotype. No patient had the ancestral hemochromatosis haplotype associated with HFE mutations.

The geographical origin of patients with or without the His63Asp mutation was similar, as follows: 88.4% and 87.5%, respectively, were from Northern Italian regions. No correlation was found between the presence of HCV infection or alcohol abuse and HFE mutations, as follows: antibodies against HCV infection, usually associated with viremia, were present in 82.3% of patients carrying the His63Asp mutation versus 73.5% of those homozygous for the absence of the mutation. Also alcohol abuse had a similar frequency in the two groups (52.9% vs. 55.8%). The prevalence of HCV infection and alcohol abuse in the three categories of iron status was not significantly different.

DISCUSSION

The clinical manifestations of sporadic PCT seem to occur in individuals following exposure to triggering agents, including iron overload, which may cause a reduction of hepatic URO-D. However, the nature of this predisposition and whether it is inherited are still a matter for debate. A genetic contribution to the iron overload frequently observed in PCT has been hypothesized in several studies and it was suggested that the hypothetical inherited predisposition for PCT could be coincidental to an inherited condition which causes iron overload.¹⁰⁻¹³ A recent report describing the high prevalence of a strong candidate causative mutation for hemochromatosis in a novel major histocompatibility class I-like gene seemed to confirm the relationship between PCT and hemochromatosis; inheritance of one or two copies of the Cys282Tyr mutation of HFE is an important susceptibility factor for sporadic PCT¹⁸ in British patients.

Our data, obtained in a large group of Italian patients with sporadic PCT, confirm a role for a HFE-linked determinant in PCT, but surprisingly do not indicate a direct association between PCT and the mutation responsible for the typical form of hemochromatosis. The Cys282Tyr mutation, strongly associated with hemochromatosis in Northern European countries and present in 44% of British patients with PCT, is rare in Italian patients with PCT, while the second known mutation of HFE, His63Asp, is highly prevalent in Italian patients with PCT, as it is present in half of the patients. Because HCV infection is frequent in Italian PCT patients and is rare in PCT patients from Northern Europe, we examined a control group of patients with HCV-chronic active hepatitis without PCT to rule out a possible association between the His63Asp mutation and HCV infection. The prevalence of HFE mutations in this group was almost identical to that observed in controls from the general population, suggesting that, in our series, the increased prevalence of His63Asp is, indeed, associated with PCT.

The role of His63Asp in hemochromatosis has not been clearly shown. Some authors suggest that His63Asp could be

a polymorphism or a polymorphic marker of another causative mutation of HFE which is different from Cys282Tyr^{16,17}; however, because the two mutations are in complete linkage disequilibrium, an analysis that considers only chromosomes "at risk," i.e., those that do not carry the Cys282Tyr, revealed also that the mutation His63Asp was overrepresented in hemochromatosis and PCT patients,³² although homozygosity for His63Asp is rare in classic hemochromatosis and the mutation is frequent in normal individuals.

Thus, several lines of evidence indicate that the His63Asp mutation could cause a more subtle abnormality of iron metabolism than Cys282Tyr. This alteration seems unable to induce the severe iron overload that is typical of hemochromatosis, even when present in the homozygous state; however, the alteration may result in the hepatocellular accumulation of toxic iron species, which accelerates the inactivation of hepatic uroporphyrinogen decarboxylase and the development of the clinical manifestations of PCT. In our series, the presence of His63Asp did not seem to correlate with the iron status of PCT patients, as judged by transferrin saturation, by iron removal via phlebotomy, and by liver iron concentration. This suggests the inability of standard parameters of iron status to consistently identify the abnormality of iron metabolism which is induced by His63Asp. HCV and/or heavy alcohol intake, which is highly prevalent in our population, might have a synergistic effect with the His63Asp mutation in inducing a clinically manifest PCT. In contrast, the Cys282Tyr mutation, which is more prevalent in Northern Europe and which causes the typical iron-storage disease, could more efficiently trigger PCT in the absence of viral liver disease.

At least a third unidentified HLA-linked genetic determinant seems to influence the iron status of Italian patients with PCT, as an increased frequency of the ancestral hemochromatosis haplotype was observed in patients with iron overload. Interestingly, none of the chromosomes bearing the ancestral haplotype carried the Cys282Tyr HFE mutation. Roberts et al.¹⁸ also found four chromosomes that carry the ancestral haplotype in the absence of the Cys282Tyr mutation in patients with PCT. Previous studies in Australian and Italian patients with hemochromatosis suggest a role for the ancestral haplotype in determining the severity of phenotype expression^{30,33} and the presence of 6 *p*-linked modifying gene(s) that can explain the haplotype-related variability of phenotype expression has been hypothesized.¹⁶

Our findings identify a state of morbidity which is associated with heterozygosity or homozygosity for His63Asp in the absence of Cys282Tyr. However, because the function of HFE and the range of clinical effects of HFE mutations have not been clearly outlined, the pathogenetic mechanism linking the His63Asp mutation with PCT remains hypothetical. In particular, it is unclear in our population whether the abnormality of iron metabolism induced by His63Asp might interfere with URO-D activity directly, or indirectly through a synergistic effect with the damage induced by viral hepatitis. The increased frequency of the ancestral hemochromatosis haplotype in patients with PCT and with iron overload suggests that, besides the existence of the HFE His63Asp mutation, also a hemochromatosis determinant distinct from Cys282Tyr could play a role in a proportion of Italian patients with both PCT and iron overload. Because HFE is not an iron binding protein, it is possible that other gene(s) that interact

with HFE might be involved in the regulation of iron absorption.

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