Iron overload in porphyria cutanea tarda

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ABSTRACT

Background and Objective. Porphyria cutanea tarda (PCT) is a disorder of porphyrin metabolism associated with decreased activity of uroporphyrinogen decarboxylase (URO-D) in the liver. The relevance of iron in the pathogenesis of PCT is well established: iron overload is one of the factors that trigger the clinical manifestations of the disease and iron depletion remains the cornerstone of therapy for PCT. A role for genetic hemochromatosis in the pathogenesis of iron overload in PCT has been hypothesized in the past but only after the recent identification of the genetic defect causing hemochromatosis has the nature of this association been partially elucidated. This review will outline current concepts of the pathophysiology of iron overload in PCT as well as recent contributions to the molecular epidemiology of hemochromatosis defects in PCT.

Evidence and Information Sources. The authors of the present review have a long-standing interest in the pathogenesis, etiology, and epidemiology of iron overload syndromes. Evidence from journal articles covered by the Science Citation Index® and Medline® has been reviewed and collated with personal data and experience.

State of the Art and Perspectives. Mild to moderate iron overload plays a key role in the pathogenesis of PCT. The recent identification of genetic mutations of the hemochromatosis gene (HFE) in the majority of patients with PCT confirms previous hypotheses on the association between PCT and hemochromatosis, allowing a step forward in the understanding of the pathophysiology of the disturbance of iron metabolism in the liver of PCT patients, and provides an easily detectable genetic marker which could have a useful clinical application. Besides the epidemiological relevance of the association between PCT and hemochromatosis mutations, a recent publication supports the idea that an enzymatic defect inherited as an autosomal recessive trait could confer a susceptibility for sporadic PCT.4 Several trigger factors would be able to interact with this latent condition to make PCT clinically manifest. Triggers factors include exposure to halogenated hydrocarbons, an increased alcohol consumption, the administration or increased endogenous production of oestrogens, liver iron overload, and infection with hepatitis viruses.1,9-12 All of these factors can cause liver disease which is almost always present in sporadic PCT.2 Altered liver enzymes are observed in the majority of patients with sporadic PCT, with liver siderosis in 75%.13

Iron and porphyria cutanea tarda

Both familial and sporadic PCT, as well as the analogous experimental uroporphyrinas in rodents14 are iron-dependent disorders.15 The association of PCT with iron overload has been known for decades.16 Independently from the cause of liver disease, the majority of patients with sporadic PCT have liver...
siderosis, increased body iron stores, and biochemical evidence of iron overload. Liver siderosis is rarely severe, reaching the lower end of the hemochromatosis range in less than 10% of patients. As initially observed by Lundvall, iron depletion by repeated phlebotomy may induce a remission of cutaneous lesions and an improvement of liver function tests, whereas replenishment of iron stores leads to relapse. This observation has been used to sustain a causal relationship between the amount of stored iron and the clinical manifestation of PCT. However, as already observed by Lundvall, phlebotomy is often clinically beneficial also in patients without biochemical or histologic evidence of iron overload. It can therefore be hypothesized that, even in the absence of evident systemic iron overload, modification of iron homeostasis in hepatocytes, possibly with accumulation of toxic iron species, could contribute to the reduced activity of URO-D.

The nature of the association between iron and PCT has not been elucidated and it is not clear how iron may trigger the clinical manifestations of the disease. Though it has been reported that iron could inhibit URO-D activity in an animal model, this appears to happen only at high and non-physiologic concentrations and it is presently accepted that iron does not directly inhibit URO-D though is required for inactivation. In vitro experiments suggest that iron may facilitate the oxidation of uroporphyrinogen and of other porphyrinogens and that the products of oxidation could inhibit the decarboxylation catalyzed by URO-D. Thus, iron could initially promote the oxidative modification of porphyrinogens which can inhibit the activity of URO-D, leading in turn to an accumulation of porphyrinogens. It has been proposed that the induction of the enzyme ALA-synthase by iron could also participate in the accumulation of porphyrinogens.

Removal of excess iron promptly induces a remission of cutaneous lesions, associated with a reduced excretion of urinary porphyrins. There is also evidence of a persistent increase in URO-D activity after iron depletion and clinical improvement was observed during four years of follow-up in four patients with sporadic PCT. It is interesting that a similar experiment in patients with familial PCT failed to cause an increase of hepatic URO-D activity, despite the fact that, also in this case, iron depletion was followed by clinical and biochemical improvement.

The causes of iron overload in patients with PCT appear to be heterogeneous. An altered iron status may be secondary to exogenous factors such as alcohol or dietary or other sources of excess iron. Chronic viral hepatitis may induce increased deposition of iron in the liver and increased deposits of hepatocyte iron have been observed by electron microscopy in biopsy specimens from patients with chronic hepatitis C. In 1992, after the identification of hepatitis C virus as the common agent of parenterally transmitted non-A, non-B hepatitis and the introduction of diagnostic assays for this agent, hepatitis C virus was recognized as being the most common etiological agent of hepatitis in Italy and in other Southern-European countries. However, it is the hypothesis of a genetically determined primary iron overload that has attracted more attention in the last two decades.

Genetic hemochromatosis and porphyria cutanea tarda

Genetic hemochromatosis is a common iron loading disease in Caucasian populations. It is inherited as an autosomal recessive trait and is characterized by an inappropriately increased absorption of dietary iron, resulting in excess iron deposition in several tissues and organs. Homozygosity for hemochromatosis occurs in 3-5 persons per 1000 and the carrier frequency is 1 in 10 to 1 in 15. Thus, it is possibly the most common inherited monogenic disorder in people of European descent. Heterozygotes for hemochromatosis do not usually have clinical signs but a recent study of clinical and biochemical abnormalities in subjects heterozygous for hemochromatosis shows that, as compared with normal individuals, they have mild but significant alterations of iron parameters. It appears therefore that about 10% of the general European population may have a genetically determined mild or latent alteration of their iron status.

After the identification of a tight linkage between hemochromatosis and the major histocompatibility complex, and in particular with the HLA-A locus, several studies provided indirect evidence of an association between PCT and hemochromatosis through analysis of HLA-A alleles in patients. A significantly increased frequency of HLA-A3 in patients with sporadic PCT was reported in some studies. These studies even led to the hypothesis that a supposed inherited predisposition for PCT could be coincident with the inherited condition causing iron overload. Other studies, however, failed to identify an association between PCT and HLA-A3. In a recent Italian study the frequency of HLA-A3 was not significantly increased in PCT patients as compared to controls; when only the subset of PCT patients with iron overload was considered, however, the frequency of HLA-A3 was found to be 42% versus 10% in PCT patients without iron overload and 22% in controls from the general population. In the last indirect study performed before the identification of the candidate gene for hemochromatosis, the relationship between HLA and sporadic PCT in British patients was investigated by means of highly polymorphic DNA markers located on the short arm of chromosome 6, telomeric to the HLA-A locus. A particular combination of these markers (haplotype) has been found to be closely associated with hemochromatosis, and is thought to
represent the ancestral hemochromatosis chromosome.45,46 The results of the British study indicate that there is a significantly increased frequency of heterozygosity for hemochromatosis in patients with PCT: up to 37% of British patients with PCT carry the ancestral hemochromatosis haplotype whereas approximately 10% of the general population do. In summary, studies performed by HLA typing do not provide evidence supporting the hypothesis that PCT could represent a peculiar presentation of hemochromatosis, but they suggest that a relevant proportion of patients with sporadic PCT may be heterozygous carriers of the disease.

**Mutations of the hemochromatosis gene in porphyria cutanea tarda**

A candidate gene for hemochromatosis encoding a HLA class I-like molecule, HFE, was recently identified.47 A missense mutation of HFE (Cys282Tyr) was found to be closely associated with the classical hemochromatosis phenotype, being present in the homozygous state in the large majority of patients of Northern-European descent.47-50 A second mutation (His63Asp) was also found to occur with an increased frequency on hemochromatosis chromosomes not bearing the Cys282Tyr mutation, but its relationship with hemochromatosis has not been clearly established.47,48 Some authors suggested that His63Asp could be a polymorphism, or a polymorphic marker of another causative mutation of HFE, different from Cys282Tyr.49,50 However, since the two mutations are in complete linkage disequilibrium, analysis considering only chromosomes at risk, i.e. those not carrying the Cys282Tyr, revealed that also the His63Asp mutation was over-represented in hemochromatosis patients although homozygosity for His63Asp is rare in classic hemochromatosis and the mutation, also in the homozygous state, is frequent in normal individuals. The analysis of compound heterozygotes for HFE gene mutations revealed that these individuals may have a higher risk of iron overload or genetic hemochromatosis than single heterozygotes for the Cys282Tyr mutation.51 The recent description of an interaction between HFE and transferrin receptor and of the effect of both HFE mutations in increasing the receptor affinity for ligand binding not only associates HFE with the regulation of transmembrane iron transport and suggests a pathologic model for hemochromatosis, but also provides a functional role for the His63Asp mutation in the disturbance of iron metabolism. While the Cys282Tyr HFE protein is retained in the endoplasmic reticulum and middle Golgi compartment, is subject to accelerated degradation, and is not presented on the cell surface, His63Asp HFE appears to undergo normal cellular processing, to be exposed on the cell surface and to complex with the transferrin receptor. The His63Asp HFE, however, is unable to decrease the affinity of the transferrin receptor for transferrin.53

The availability of a genetic marker for hemochromatosis allowed direct investigation of its relationship with PCT. Data on the prevalence of HFE mutations in PCT patients are reported in Table 1. A significantly increased frequency of the Cys282Tyr HFE mutation in PCT patients was reported for the first time by Roberts et al.55 in a British series, confirming that inheritance of one or more hemochromatosis genes is an important susceptibility factor for sporadic PCT. The mutation was found on 30% of alleles from PCT patients versus 5.9% alleles from controls. The frequency of the second mutation of HFE was not found to be increased. Similar data were described in a study performed in Australia.56 Surprisingly different results were obtained in Italy by analysis of HFE genotypes in male patients with PCT.57 The data did not confirm an association of PCT with the mutation strongly associated with hemochromatosis in Northern European countries; the second mutation of HFE, His63Asp, however, had a significantly increased frequency, being present in half of the patients. An increased frequency of both HFE mutations in PCT patients is also described by two studies performed in the Netherlands and in the United States, though the small number of patients on whom the mutational analysis of HFE was performed did not allow a statistical comparison with a normal control population.58,59 Three major factors may account for some of the differences between these studies: 1) the main hemochromatosis mutation is less frequent in Southern-European countries than in Britain, Australia, and the North-American community of British ancestry; 2) hemochromatosis appears to be less genetically homogeneous in Southern Europe than it is in Great Britain and in Australia. The Cys282Tyr mutation was present in the homozygous state in about 90% of British patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Cys282Tyr</th>
<th>His63Asp</th>
<th>Patients with HFE mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Great Britain</td>
<td>41</td>
<td>44% (11%)</td>
<td>31% (29%)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>15</td>
<td>67% (17%)</td>
<td>33% (n.a.)</td>
</tr>
<tr>
<td>Italy</td>
<td>68</td>
<td>3% (1.5%)</td>
<td>51% (24%)</td>
</tr>
<tr>
<td>United States</td>
<td>26</td>
<td>42% (n.a.)</td>
<td>33% (n.a.)</td>
</tr>
<tr>
<td>Australia</td>
<td>27</td>
<td>44% (12%)</td>
<td>44% (n.a.)</td>
</tr>
</tbody>
</table>

Legend: n.a. = not available.
and in 100% of Australian patients with overt hemochromatosis, but in less than 70% in Italy, and in Southern France. The distribution of factors able to 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, being present in 70-90% of patients, while it is rare in Northern-European countries where alcohol is the prevalent etiological agent for chronic liver disease associated with PCT. Hepatitis C virus might have a synergistic effect with both HFE mutations in inducing a clinically manifest PCT. In contrast, the Cys282Tyr mutation, more prevalent in Northern Europe and causing the typical iron-storage disease, could more efficiently trigger PCT in the absence of viral liver disease. Another finding reported in two of these studies is that the presence of HFE mutations in PCT patients as evaluated by transferrin saturation, iron removed by phlebotomy and liver iron concentration. This suggests that the standard parameters of iron status might be unable to identify consistently the mild abnormality of iron metabolism induced by HFE mutations. It can be hypothesized that the metabolic abnormality associated with HFE mutations may result in hepatocellular accumulation of toxic iron species, capable of promoting the inactivation of hepatic uroporphyrinogen decarboxylase and the development of the clinical manifestations of PCT.

The relevance of other genetic and acquired factors in inducing iron overload in PCT is suggested by two observations: 1) despite the high frequency of HFE mutations in PCT patients, a considerable proportion of patients did not carry HFE mutations, and some patients had the ancestral hemochromatosis haplotype in the absence of HFE mutations. It is possible that at least a third unidentified genetic determinant may influence the iron status of PCT patients where the existence of other genetic determinants capable of influencing the iron status and unlinked to chromosome 6p remains to be proved; 2) among Italian PCT patients the large majority of subjects, including those carrying HFE mutations, have one or more acquired factors potentially able to alter iron status, such as viral hepatitis or alcohol abuse. Thus it is likely that in most cases the presence of HFE mutations contributes to the inactivation of URO-D through interaction with other factors capable of altering the iron homeostasis in the liver. This appears to be particularly true for the H63Asp mutation which, according to all the available evidence, causes a mild defect of iron metabolism.

Conclusions

Several lines of evidence indicate that PCT is an iron dependent disorder. Mild to moderate systemic or liver iron overload is present in the majority of patients, and a therapy based on iron depletion by repeated phlebotomy controls symptoms effectively also in patients without evident signs of altered iron status. Iron depletion may also be clinically effective in the rare familial form of PCT in which it is likely that excess hepatocyte iron may contribute to inactivating the residual enzyme.

The hypothesis of an association between PCT and genetic hemochromatosis has been strengthened by the identification of HFE, the gene involved in hemochromatosis, since 53-87% of patients with sporadic PCT carry mutant alleles of this gene. However, since the function of HFE and the range of clinical effects of HFE mutations have not been completely outlined, the detailed pathogenetic mechanism linking mutations of this gene with PCT remains hypothetical. In particular, it is still unclear whether the abnormality of iron metabolism induced by HFE mutations might interfere with URO-D activity directly or through a synergistic effect with a damage induced by viral hepatitis or other factors. Availability of a genetic marker for hemochromatosis will allow some of these issues to be addressed by studying aspects of porphyrins and iron metabolism in liver samples obtained from patients with PCT, liver disease of different etiology and different HFE genotypes and by in vitro studies on genotyped cells and tissues.

Contributions and Acknowledgments

SF was the principal investigator and designed the study. MS was responsible for writing the paper. All the authors contributed to the analysis and discussion of data.

Disclosures

Conflict of interest: none.

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References

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