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Iron overload in porphyria cutanea tarda

MAURIZIO SAMPIETRO, GEMINO FIORELLI, SILVIA FARGION
Dipartimento di Medicina Interna, Università di Milano, IRCCS Ospedale Maggiore Policlinico, Milan, Italy

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, allows 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, however, it remains to be fully understood how iron overload, and in particular the cellular modifications of the iron status secondary to hemochromatosis mutations, affect the activity of URO-D, and how the altered iron metabolism interacts with the other two common triggers for PCT and etiological agents for the associated liver disease: alcohol and hepatitis viruses. The 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.**

Key words: porphyria, porphyria cutanea tarda, iron, hemochromatosis, HFE, hepatitis, liver disease

Correspondence: Prof. Silvia Fargion, Dipartimento di Medicina Interna, Pad. Granelli, IRCCS Policlinico, via F. Sforza 35, 20122 Milan, Italy. Phone: international +39-02-55033757 – Fax: international +39-02-55180241 – E-mail: silvia.fargion@unimi.it

Porphyria cutanea tarda (PCT),[1] a skin disorder usually presenting with a vesiculo-bullous eruption on the hands and face, scarring and hirsutism, is caused by photosensitization induced by the deposition of porphyrins in the skin. It is the most common type of porphyria and is caused by a reduced activity of hepatic uroporphyrinogen decarboxylase (URO-D) in the liver.[2] The reduced enzymatic activity of URO-D leads to the accumulation of uroporphyrinogen and other porphyrinogen substrates of URO-D, and increased oxidation of porphyrinogens to porphyrins. Familial PCT, caused by mutations of the URO-D gene[3] able to reduce URO-D activity in all tissues, is rare and is inherited as an autosomal dominant trait.[4] In sporadic PCT URO-D activity is reduced in hepatocytes through inactivation of a normal enzyme[5] while a normal activity is found in erythrocytes, in cultures of skin fibroblasts[6,7] and, apparently, in all other cells and tissues.[1] It has been hypothesized that an enzymatic defect inherited as an autosomic recessive trait could confer a susceptibility for sporadic PCT.[7,8] Several trigger factors would be able to interact with this latent condition to make PCT clinically manifest. Trigger 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 at presentation; a histopathologic picture of chronic hepatitis is observed in over 80% of patients and liver siderosis in 75%.[13]

Iron and porphyria cutanea tarda

Both familial and sporadic PCT, as well as the analogous experimental uroporphyrias in rodents[14] 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.[9] Liver siderosis is rarely severe, reaching the lower end of the hemochromatosis range in less than 10% of patients.[9,17] As initially observed by Lundvall[18] 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.[19] This observation has been used to sustain a causal relationship between the amount of stored iron and the clinical manifestation of PCT.[20] However, as already observed by Lundvall,[18] 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.[21]

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,[22] 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.[23] *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.[22,24]

Removal of excess iron promptly induces a remission of cutaneous lesions,[18] 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.[7] 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.[4]

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[25] or dietary or other sources of excess iron.[1] Chronic viral hepatitis may induce increased deposition of iron in the liver[26,27] and increased deposits of hepatocyte iron have been observed by electron microscopy in biopsy specimens from patients with chronic hepatitis C.[28] 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[12,29] and in other Southern-European countries.[30, 31] 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.[32] It is inherited as an autosomal recessive trait[33] 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.[34,35] 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[36] 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[37] 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.[17,38,39] These studies even led to the hypothesis that a supposed inherited predisposition for PCT could be coincident with the inherited condition causing iron overload.[20] Other studies, however, failed to identify an association between PCT and HLA-A3.[40-43] In a recent Italian study the frequency of HLA-A3 was not significantly increased in PCT patients as compared to in 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.[21] 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.[44] 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[51] 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.[52] 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[53] 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,[54] 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;[60] 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 1. Frequency of carriers of HFE mutations among patients with porphyria cutanea tarda. The frequency of HFE mutations in control individuals from the same population is reported in brackets.

	Patients	*Cys282Tyr*	*His63Asp*	*Patients with HFE mutations*	*Ref.*
Great Britain	41	44% (11%)	31% (29%)	68% (37%)	55
The Netherlands	15	67% (17%)	33% (n.a.)	87% (n.a.)	58
Italy	68	3% (1.5%)	51% (24%)	53% (25%)	57
United States	26	42% (n.a.)	31% (n.a.)	73% (n.a.)	59
Australia	27	44% (12%)	44% (n.a.)	70% (n.a.)	56

Legend: n.a. = not available.

and in 100% of Australian patients with overt hemochromatosis,[49,55] but in less than 70% in Italy,[61,62] and in Southern France;[63] 3) 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[12,30,31] being present in 70-90% of patients, while it is rare in Northern-European countries[64,65] 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[57,59] is that the presence of His63Asp did not appear to correlate with the iron status of 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 His63Asp. It can be hypothesized that the metabolic abnormality associated with His63Asp 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.[55,57] It is possible that at least a third unidentified HLA-linked genetic determinant may influence the iron status of PCT patients while 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 His63Asp 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

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References

1. Kappas A, Sassa S, Galbraith RA, Nordmann Y. The porphyrias. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The molecular and metabolic basis of inherited disease. 7th ed. New York: McGraw-Hill, 1995. p. 2103-59.
2. De Verneuil H, Aitken G, Nordmann Y. Familial and sporadic porphyria cutanea tarda: two different diseases. Hum Genet 1978; 44:145-51.
3. Garey JR, Hansen JL, Kushner JP. A point mutation in the coding region of uroporphyrinogen decarboxylase associated with familial porphyria cutanea tarda. Blood 1989; 73:892-5.
4. Kushner JP, Barbuto AJ, Lee GR. An inherited enzymatic defect in porphyria cutanea tarda: decreased uroporphyrinogen decarboxylase activity. J Clin Invest 1976; 58:1089-97.
5. Garey JR, Franklin KF, Brown DA, Harison LM, Metcalf KM, Kushner JP. Analysis of uroporphyrinogen decarboxylase complementary DNAs in sporadic porphyria cutanea tarda. Gastroenterology 1993; 105:

165-9.
6. Elder GH, Lee GB, Tovey JA. Decreased activity of hepatic uroporphyrinogen decarboxylase in sporadic porphyria cutanea tarda. N Engl J Med 1978; 299: 274-8.
7. Elder GH, Urquhart AJ, De Salamanca RE, Munoz JJ, Bonkovsky HL. Immunoreactive uroporphyrinogen decarboxylase in the liver in porphyria cutanea tarda. Lancet 1985; 1:229-32.
8. Hindmarsh JT. Enzyme heterogeneity in the porphyria. Clin Biochem 1990; 23:371-4.
9. Lundvall O, Weinfeld A, Lundin P. Iron storage in porphyria cutanea tarda. Acta Med Scand 1970; 188:37-53.
10. Haberman HF, Rosemberg L, Menon LA. Porphyria cutanea tarda: comparison of cases precipitated by alcohol and estrogens. Can Med Assoc J 1975; 113: 633-55.
11. Rocchi E, Gibertini P, Cassanelli M, Pietrangelo A, Jensen J, Ventura E. Hepatitis B virus infection in porphyria cutanea tarda. Liver 1986; 6:153-7.
12. Fargion S, Piperno A, Cappellini MD, et al. Hepatitis C virus and porphyria cutanea tarda: evidence of a strong association. Hepatology 1992; 16:1322-6.
13. Lefkowitch JK, Grossman ME. Hepatic pathology in porphyria cutanea tarda. Liver 1983; 3:19-29.
14. Smith AG, Francis JE. Genetic variation of iron-induced uroporphyria in mice. Biochem J 1993; 291: 29-35.
15. Elder JH. Porphyria cutanea tarda: a multifactorial disease. In: Champion RH, Pye RJ, eds. Recent advances in dermatology. 8. Edinburgh: Churchill Livingstone, 1990. p. 55-70.
16. Berlin SO, Brante G. Iron metabolism in porphyria and haemochromatosis. Lancet 1962; 2:729.
17. Edwards CQ, Griffen LM, Goldgar DE, Skolnick MH, Kushner JP. HLA-linked hemochromatosis alleles in sporadic porphyria cutanea tarda. Gastroenterology 1989; 97:972-81.
18. Lundvall O. The effect of phlebotomy therapy in porphyria cutanea tarda. Acta Med Scand 1971; 189:33-49.
19. Lundvall O. The effect of replenishment of iron stores after phlebotomy therapy in porphyria cutanea tarda. Acta Med Scand 1971; 189:51-63.
20. Adams PC, Powell LW. Porphyria cutanea tarda and HLA-linked hemochromatosis – All in the family? Gastroenterology 1987; 92:2033-5.
21. Fargion S, Fracanzani AL, Romano R, et al. Genetic hemochromatosis in Italian patients with porphyria cutanea tarda: possible explanation for iron overload. J Hepatol 1996; 24:564-9.
22. Bonkovsky HL. Mechanism of iron-potentiation of hepatic uroporphyria: studies in cultured chicken embryo liver cells. Hepatology 1989; 10:354-64.
23. Elder GH, Roberts AG. Uroporphyrinogen decarboxylase. J Biomembr Bioenerg 1995; 27:207-14.
24. Bonkovsky HL, Sinclair PR, Sinclair JF. Hepatic heme metabolism and its control. Yale J Biol Med 1979; 52:13-37.
25. Chapman RW, Morgan MY, Laulicht M, Hoffbrand AV, Sherlock S. Hepatic iron stores and markers of iron overload in alcoholics and patients with hemochromatosis. Dig Dis Sci 1982; 27:909-16.
26. Di Bisceglie AM, Axiotis CA, Hoofnagle JH, Bacon BR. Measurements of iron status in patients with chronic hepatitis. Gastroenterology 1992; 102:2108-13.
27. Piperno A, D'Alba R, Fargion S, et al. Liver iron concentration in chronic viral hepatitis: a study of 98 patients. Eur J Gastroenterol Hepatol 1995; 7:1203-8.
28. Isomura T, Yano M, Hayashi H, Sakamoto N. Excess iron in the liver of patients with chronic hepatitis C. J Clin Electron Microsc 1992; 25:231-7.
29. Piperno A, D'Alba R, Roffi L, et al. Hepatitis C virus infection in patients with idiopatic hemochromatosis and porphyria cutanea tarda. Arch Virol 1992; 4:215-6.
30. Lacour JP, Bodokh I, Castanet J, Bekri S, Ortonne JP. Porphyria cutanea tarda and antibodies to hepatitis C virus. Br J Dermatol 1993; 128:121-3.
31. Herrero C, Vicente A, Bruguera M, et al. Is hepatitis C virus infection a trigger of porphyria cutanea tarda? Lancet 1993; 341:788-9.
32. Tavill AS, Bacon BR. Hemochromatosis: iron metabolism and the iron overload syndromes. In: Zakim D, Boyer T, eds. Hepatology. A textbook of liver disease. Philadelphia: Saunders & Co., 1990. p. 1273-99.
33. Simon M, Bourel M, Genetet B, Fauchet R. Idiopathic hemochromatosis: demonstration of recessive transmission and early detection by family HLA typing. N Engl J Med 1977; 297:1017-21.
34. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. N Engl J Med 1988; 318:1355-62.
35. Velati C, Piperno A, Fargion S, Colombo S, Fiorelli G. Prevalence of idiopathic hemochromatosis in Italy: study of 1301 blood donors. Haematologica 1990; 75:309-12.
36. Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP. Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. N Engl J Med 1996; 335:1799-805.
37. Simon M, Bourel M, Fauchet R, Genetet B. Association of HLA-A3 and HLA-B14 antigens with idiopathic haemochromatosis. Gut 1976; 17:332-4.
38. Kuntz BME, Goerz G, Merk H, Strohmeyer G, Bruster HT. HLA-A3 and B7 in porphyria cutanea tarda. Tissue Antigens 1984; 24:67-9.
39. Kushner JP, Edwards CQ, Dadone MM, Skolnick MH. Heterozygosity for HLA-linked hemochromatosis is a likely cause of hepatic siderosis associated with porphyria cutanea tarda. Gastroenterology 1985; 88: 1232-8.
40. Santoianni P, De Felice M, Ayala F, Budillon G, Zappacosta S. A novel association between HLA and disease: porphyria cutanea tarda and HLA-AW32. Dermatologica 1980; 160:371-5.
41. Llorente L, de Salamanca RE, Campillo F, Pena ML. HLA and porphyria cutanea tarda. Arch Dermatol Res 1980; 269:209-10.
42. Kostler VE, Gebhardt B, Seebacher C. HLA system und porphyria cutanea tarda. Dtsch Z Verdau Stoffwechselkr 1984; 44:95-100.
43. Beaumont C, Fauchet R, Phung LN, Verneuil HD, Gueguen M, Nordmann Y. Porphyria cutanea tarda and HLA-linked hemochromatosis: evidence against a systematic association. Gastroenterology 1987; 92: 1833-8.
44. Roberts AG, Whatley SD, Nicklin S, et al. The frequency of haemochromatosis-associated alleles is increased in British patients with sporadic porphyria cutanea tarda. Hepatology 1997; 25:159-61.
45. Raha-Chowdhury, Bowen DJ, Stone C, et al. New polymorphic microsatellite markers place the haemochromatosis gene telomeric to D6S105. Hum Mol Genet 1995; 4:1869-74.
46. Camaschella C, Piperno A. Hereditary hemochromatosis: recent advances in molecular genetics and clinical management. Haematologica 1997; 82:77-84.
47. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13:399-408.

48. Beutler E, Gelbart T, West C, et al. Mutation analysis in hereditary hemochromatosis. Blood Cells Mol Dis 1996; 22:187-94.
49. Jazwinska EC, Cullen LM, Busfield F, et al. Haemochromatosis and HLA-H. Nat Genet 1996; 14:249-51.
50. Jouanolle AM, Gandon G, Jézéquel P, et al. Haemochromatosis and HLA-H. Nat Genet 1996; 14:251-2.
51. Beutler E. Genetic irony beyond haemochromatosis: clinical effects of HLA-H mutations. Lancet 1997; 349: 296-7.
52. Martinez PA, Biron C, Blanc F, et al. Compound heterozygotes for hemochromatosis gene mutations: may they help to understand the pathophysiology of the disease? Blood Cells Mol Dis 1997; 23:269-76.
53. Feder JN, Penny DM, Irrinki A, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci USA 1998; 95:1472-7.
54. Waheed A, Parkkila S, Zhou XY, et al. Hereditary hemochromatosis: effects of C282Y and H63D mutations on association with beta2-microglobulin, intracellular processing, and cell surface expression of the HFE protein in COS-7 cells. Proc Natl Acad Sci USA 1997; 94:12384-9.
55. Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH. Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. Lancet 1997; 349:321-3.
56. Stuart KA, Busfield F, Jazwinska EC, et al. The C282Y mutation in the hemochromatosis gene (HFE) and hepatitis C virus infection are independent cofactors for pophyria cutanea tarda in Australian patients. J Hepatol 1998; 28:404-9.
57. Sampietro M, Piperno A, Lupica L, et al. High prevalence of the His63Asp HFE mutation in Italian patients with porphyria cutanea tarda. Hepatology 1998; 27: 181-4.
58. Santos M, Clevers HC, Marks JJM. Mutations of the hereditary hemochromatosis candidate gene HLA-H in porphyria cutanea tarda. N Engl J Med 1997; 336: 1327-8.
59. Bonkowsky HL, Poh-Fitzpatrick M, Pimstone M, et al. Porphyria cutanea tarda, hepatitis C, and HFE gene mutations in North America. Hepatology 1998:1661-9.
60. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJH. Global prevalence of putative haemochromatosis mutations. J Med Genet 1997; 34:275-8.
61. Carella M, D'Ambrosio L, Totaro A, et al. Mutation analysis of the HLA-H gene in Italian hemochromatosis patients. Am J Hum Genet 1997; 60:828-32.
62. Piperno A, Sampietro M, Pietrangelo A, et al. Heterogeneity of hemochromatosis in Italy. Gastroenterology 1998; 114:996-1002.
63. Borot N, Roth MP, Malfroy L, et al. Mutation in the MHC class I-like candidate gene for hemochromatosis. Immunogenetics 1997; 45:320-4.
64. Murphy A, Dooley S, Hillary IB, Murphy GM. HCV infection in porphyria cutanea tarda. Lancet 1993; 341:1534-5.
65. Stölzel U, Kostler E, Koszka C, et al. Low prevalence of hepatitis virus infection in porphyria cutanea tarda in Germany. Hepatology 1995; 21:1500-3.