Hemojuvelin and Hepcidin Gene Mutations in Patients with Porphyria Cutanea Tarda from Southern France

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Accepted January 21, 2010.

Porphyria cutanea tarda (PCT), the most frequent subset of porphyria in western countries, is related to decreased activity of one of the key enzymes of haem synthesis, uroporphyrinogen decarboxylase (UROD) (1). Several well-known, often combined, factors may lead to overt PCT, including hepatitis C (HCV) infection, exposure to hepatotoxic drugs, alcohol abuse and liver iron overload. This last factor's pathological mechanisms in PCT may result partly from mutations affecting the various genes involved in the regulation of iron metabolism. Mutations in the *HFE* gene (C282Y and H63D) have been identified in PCT at an allele frequency up to 30% in several studies (1–4). Other studies have investigated additional genes involved in iron metabolism, namely the transferrin-receptor type 2 (*TFR2*) or the cytochrome P450 (*CYP1A2*), but results have remained inconclusive to date (5, 6).

Mutations of other genes involved in iron metabolism should also be considered. Recently, new relevant biochemical actors have been identified in rare subsets of hereditary hemochromatosis (HH), i.e. the hemojuvelin (*HJV*) and hepcidin (*HAMP*) genes in juvenile haemochromatosis (JH) (7). Hemojuvelin protein has clearly been involved in the regulation of the expression of hepcidin, a key protein of iron metabolism also involved in JH (8). Hepcidin is an antimicrobial peptide that indirectly decreases intestinal iron absorption (9). To investigate the possible involvement in PCT of deleterious mutations of these two genes and their possible relationship with iron overload, a retrospective study was designed targeting 35 PCT patients from southern France.

PATIENTS AND METHODS

All patients with overt PCT identified in our department between 1997 and 2007 were retrospectively selected. Diagnosis of PCT was established on the basis of usual clinical and biochemical data. Familial PCT was identified based on familial history if applicable and/or on a decrease in erythrocytes UROD activity of at least 50%. For a rapid sequence analysis of the *HJV* and *HAMP* genes, a single-condition amplification method, as described by Cunat et al. (10), was applied. Additionally, the presence of the two main mutations of the *HFE* gene was also investigated, as previously described (10). A total of 102 unrelated chromosomes from individuals with no background of iron overload were used as controls. Hepatitis C and HIV status were assessed at the time of PCT diagnosis, as was serum ferritin level. Alcohol consumption was retrospectively extracted from patients' medical files.

RESULTS

A total of 35 patients were included in this study, 10 women and 25 men (sex ratio M/F 2.5). The mean age of the patients at diagnosis was 51.2 years (age range 37–75 years). A sporadic or familial subset of PCT was identified in 33 and 2 patients, respectively. No patient had a clinical pattern or a reminiscent family history of HH.

Overt iron overload, defined by an elevated serum ferritin level above 450 µg/l, was present in 10 patients (28.6%).

HCV infection gave a positive result in 17 patients, but only 8 had an active infection and were receiving INF-α therapy at the time of PCT diagnosis.

Three patients (8.57%) were compound heterozygote for *HFE* C282Y and *HFE* H63D mutations, five (14.3%) displayed a heterozygous C282Y mutation, seven (20%) had a heterozygous H63D mutation and two (5.7%) were homozygote for H63D mutation.

No sequence change was observed in the *HJV* gene. Conversely, a heterozygous intronic polymorphism located 6 bp upstream from exon 2, c.98-6C>G, was present on one allele of a single patient (allele frequency 1.42%), but was also present in 3 control chromosomes (allele frequency 2.9%).

In the same patient, a silent mutation, p.T84T, was found in the *HAMP* gene, but not among the controls. This patient had a moderately severe PCT with lesions restricted to the hands and forearms, evolving in a context of untreated HCV (with no significant viral load) and alcohol abuse. Ferritin level was normal at diagnosis. He also displayed a heterozygous *HFE* C282Y mutation.

Finally, a previously described heterozygous mutation of the *HAMP* gene, p.G71D, was identified in one patient and at an allele frequency of 0.3% in the controls. *HJV* and *HFE* genes did not display any change and ferritin level was normal. In this female patient, no HCV or HIV infection, alcohol abuse nor family history of PCT were recorded, but PCT symptoms occurred shortly after a substitutive hormonal treatment for menopause was implemented.

DISCUSSION

In our series, the single intronic polymorphism c.98-6C>G of the *HJV* gene was identified in only one pa-

tient, who did not display any significant iron overload. The allele frequency (1.42%) was not different from the controls ($p = 0.32$). This polymorphism has been described previously by Lee et al. (11) with an allele frequency of 2.03% in a group of white individuals with iron overload and it seemed to be the most frequent sequence variation of the *HJV* gene. Its impact on iron metabolism is questionable and is not supported by a large study involving 136 C282Y homozygotes in whom iron parameters in the patients harbouring this polymorphism were comparable with, or lower than those identified of the matched controls (11). Furthermore, co-inheritance of c.98-6C>G and of C282Y heterozygosity or C282Y/H63D compound heterozygosity did not display a HH phenotype or a significant iron overload in two males and two females.

On the other hand, Mendes et al. (12) considered this polymorphism as a novel putative splicing mutation resulting in an alteration of the splicing variants produced as a result.

In *HAMP*, we found two different sequence variations. The first one, the heterozygous p.T84T silent mutation, was identified in the patient presenting the c.98-6C>G substitution. This sequence variation has been reported previously by Merryweather-Clark et al. (13) in an individual with normal ferritin levels and in two individuals with slightly elevated ferritin levels. It does not introduce any change in the primary structure of the protein, although it modifies a Mae*II* restriction site. It could nevertheless play an additive role in our patient also harbouring the polymorphism of *HJV*.

The heterozygous pG71D mutation of *HAMP* reported in a second patient of our series has also been previously described by Merryweather-Clarke et al. (13). This heterozygous mutation was also found in one patient in a series of 96 PCT patients reported by Ajioka et al., (14) resulting in an allele frequency of 0.5%. However, ferritin levels were normal at the time of diagnosis in these two patients. This mutation is correlated with high ferritin levels when associated with *HFE* mutations (14,) but our patient had normal ferritin levels and had a wild-type *HFE* genotype.

By contrast with JH, neither *HJV* nor *HAMP* seem to play a significant role in PCT-associated iron overload according to our results, since no significant mutation could be identified, even in patients with the most elevated iron parameters.

Overall, these results contrast strongly with the status of *HFE*. Our data might also suggest that iron overload may be currently less important as an inducer of PCT expression in occidental countries than other factors, such as viral hepatic infections. However, new insights into the complex iron metabolism might provide new leads to follow in PCT. The sensitivity of future studies would probably be increased by screening patients with significant iron overload, as determined by liver magnetic resonance imaging (15).

The authors declare no conflict of interest.

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