

## Haemochromatosis Gene Mutations and Response to Chloroquine in Sporadic Porphyrria Cutanea Tarda

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Accepted November 11, 2005.

Sir,

Treatment of porphyria cutanea tarda (PCT) is based mainly on low-dose chloroquine and phlebotomy. The rationale for phlebotomy seems to be the reduction of iron overload. However, chloroquine therapy also reduces serum iron markers (1) and is less invasive and time-consuming than phlebotomies (2). The therapeutic decision remains empirical and there is no consensus strategy in treating PCT.

An increased frequency of the haemochromatosis gene (*HFE*) C282Y mutation in patients with PCT has been widely reported (3–6). The association between the H63D mutation and PCT has also been described in studies from the Mediterranean area (7, 8).

Few studies dealing with the response to anti-malarials and *HFE* mutations in patients with PCT have been published.

### MATERIALS AND METHODS

We retrospectively analysed a database of 65 patients with sporadic PCT (sPCT). The diagnosis of PCT was based on characteristic clinical and laboratory features. All patients had photosensitive skin lesions, marked increase of uro- and heptacarboxy-porphyrins in urine and presence of isocoproporphyrin in faeces. All patients included in the study showed a complete clinical and biochemical response after treatment. Biochemical remission was defined as normalization of urinary uroporphyrin (< 200 µg/24 h).

Forty patients (group A) had received chloroquine diphosphate (250 mg twice weekly) alone and had achieved a complete response in less than 6 months. Twenty-five patients (group B) had also received chloroquine alone initially (for at least 6 months) but due to the lack of response they had phlebotomies (0.4 l weekly for an average 1.5 months) in addition, in order to obtain the afore-mentioned biochemical remission.

In order to evaluate a possible correlation between the therapeutic response and the *HFE* mutations, C282Y and H63D mutations were tested in all patients from groups A and B. These tests were performed some months after the patients had terminated their therapeutic regimes so that the clinician (CH) was not influenced by the *HFE* genotypes when deciding a specific therapeutic regime.

Mutation analysis of the *HFE* gene was performed on DNA extracted from frozen stored samples of whole blood using the QIAamp Blood Kit (Qiagen, Santa Clarita, CA, USA) and amplified by PCR, which was followed by restriction endonuclease digestion. The restriction enzymes used were *RsaI* for exon 4 and *MboI* for exon 2, and the resulting fragments were

separated by polyacrylamide gel electrophoresis (PAGE) and visualized by ethidium bromide staining.

The significance of the differences between frequencies for patients from both groups was determined by the  $\chi^2$  test.

### RESULTS

Twenty-eight patients (28/65; 43%) were heterozygous for the H63D mutation and 7 patients were homozygous (7/65; 10.7%) for this mutation. The prevalence of the H63D mutation was similar in both groups of therapeutic regimes (group A; 57.5%; group B; 48%,  $p = 0.45$ ).

The C282Y mutation was present in heterozygosity in 11 patients (11/65; 16.9%). None of the subjects included in the study was homozygous for the C282Y mutation. Eight out of 11 patients with the C282Y mutation (73%) had received phlebotomies as well as chloroquine in order to achieve a clinical and biochemical response. Only 17 out of 54 (31.5%) patients without the C282Y mutation had received phlebotomies. When comparing both groups we found an increase in the C282Y mutation in patients from group B (those who had also received phlebotomies) (8/25; 32%) compared with group A (those on chloroquine) (3/40; 7.5%,  $p = 0.016$ ).

Compound heterozygosity (C282Y/H63D) was detected in 3 patients, 2 from group A and 1 from group B.

### DISCUSSION

Most patients with PCT have iron overload with increased plasma iron (sideraemia) and ferritin levels and hepatic siderosis similar to those observed in individuals with haemochromatosis. A high frequency of the C282Y mutation (ranging from 11% to 46%) in the *HFE* gene, has been found in patients with PCT from several countries (3–6). Several authors have found increased iron parameters in patients with PCT with C282Y homozygous and compound C282Y/H63D heterozygous status (5, 9, 10).

Treatment of PCT is based mainly on low-dose chloroquine and phlebotomies. Phlebotomies are invasive and time-consuming procedures and objective data to identify the profile of responders to chloroquine would

be extremely valuable. As far as we know only one previous study has addressed the relationship between *HFE* mutations and the response to these therapeutic modalities. Stölzel et al. (10) studied the response to chloroquine diphosphate monotherapy in a group of 62 patients with PCT and *HFE*-established genotype. The average treatment time was 16 months. In contrast to our study, Stölzel et al. found that C282Y heterozygous, compound heterozygous and wild-type *HFE* carriers showed a good response to chloroquine use with sustained remission of cutaneous lesions, decreased liver enzymes, and reduced excretion of urinary porphyrins. However, the serum iron markers decreased after treatment with chloroquine only in patients with the *HFE* wild type. On the other hand, C282Y homozygotes did not manifest any improvement and did not benefit from chloroquine use.

We observed that patients with PCT with suboptimal response to chloroquine after a 6-month treatment period showed an increased prevalence of the C282Y mutation in heterozygosity. Phlebotomies had to be added to obtain full therapeutic response in this group of patients. Nevertheless, our study was retrospective and limited by the fact that therapeutic response was assessed after a 6-month period.

Our results and those reported previously (10) seem to show a link between the C282Y *HFE* mutation, either in homozygosity or heterozygosity, and a suboptimal response to chloroquine. In conclusion, there is some evidence suggesting that phlebotomies should be the first-line therapy in patients with PCT and the C282Y *HFE* mutation. The evaluation of larger series of patients needs to be carried out in order to investigate further these preliminary results.

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