**Objective:** To examine the role of hemochromatosis (HFE) gene mutations, which are associated with porphyria cutanea tarda (PCT), in the therapeutic response to chloroquine.

**Design:** We retrospectively analyzed a database (Excel version 2001 [Microsoft Excel, Redmond, Wash]; date range of search, 1985-1999) of chloroquine-treated patients with PCT on whether HFE mutations (C282Y and H63D) might have influenced the clinical response, urinary porphyrin excretion, liver enzyme activities, and serum iron markers. Serum samples and corresponding complete sets of data before and after therapy were available in 62 of 207 patients with PCT who were treated exclusively with chloroquine.

**Settings:** Academic teaching hospital.

**Intervention:** For treatment, low-dose chloroquine diposphate, 125 to 250 mg twice weekly, was used during a median time of 16 months (range, 12-26 months).

**Results:** Of the 62 German patients with PCT, 37 (60%) carries HFE mutations. Chloroquine therapy was accompanied by clinical remission and reduced urinary porphyrin excretion (P<.001) in the 24 patients (39%) with HFE wild type as well as in 35 HFE heterozygous patients with PCT (56%). Decreases of serum iron markers following chloroquine therapy were limited to patients with PCT and HFE wild type. All patients homozygous for the C282Y mutation (3 [5%] of 62) had high serum iron, ferritin, and transferrin saturation and failed to respond to chloroquine treatment.

**Conclusions:** The therapeutic response to chloroquine was not compromised by C282Y heterozygosity and compound heterozygosity of HFE mutations. Because HFE C282Y homozygotes (++/+) did not respond to chloroquine and a decrease in serum iron concentration was limited to patients with PCT and HFE wild type, phlebotomy should be first-line therapy in patients with PCT and HFE mutations.

Arch Dermatol. 2003;139:309-313

---

**For editorial comment see page 379**

In a study from northern Italy, however, C282Y mutations occurred as frequently in patients with PCT as in controls (1.5%), whereas the H63D mutation was significantly increased in patients with PCT. In Japanese and Bulgarian patients, no C282Y mutations were found. Thus regional and national variations in prevalence of the C282Y mutation appear to contribute to the geographically different risk for the manifestation of PCT via iron accumulation.

To our knowledge, there is no information on clinical implications of HFE mu-
METHODS

PATIENTS

The study population comprised 207 patients with PCT who lived in an area of approximately 260 km² in and around Dresden, Germany, who were treated exclusively with chloroquine diphosphate (125-250 mg twice weekly) between 1985 and 1999. They were monitored in a follow-up program performed in a specialized outpatient clinic of the Department of Dermatology, Hospital Dresden-Friedrichstadt, Dresden. For the present study, 62 consecutive patients with PCT (26 women and 36 men; mean±SD [median, range] age, 51±13 [49, 21-80] years) were included based on (1) available serum samples and (2) corresponding complete sets of data before and after therapy to analyze the therapeutic response to chloroquine.

METHODS

Urinary porphyrins were separated by thin-layer chromatography and measured by spectrophotometric absorption. Urinary porphyrins were separated by thin-layer chromatography. Response to therapy was defined clinically by remission of skin lesions and characteristic patterns of urinary heme precursors. Response to therapy was defined as remission of skin lesions and characteristic patterns of urinary heme precursors.

Hepatitis virus markers were analyzed as described previously. Because our subjects lacked clinical evidence for immunodeficiency and because human immunodeficiency virus (HIV) infection is relatively rare in Saxony (former East Germany), we did not search for HIV infection (eg, by testing for anti-HIV antibodies) in these patients with PCT.

Patients were treated with chloroquine diphosphate (Chlorochin; Berlin-Chemie, Berlin, Germany), 125 to 250 mg twice weekly, with a median treatment time of 16 months (range, 12-26 months). All patients were advised to avoid alcohol. Other dietary restrictions were not recommended. During the period of chloroquine medication, neither phlebotomy nor hepatitis virus treatment was performed.

Genomic DNA was extracted from serum samples and used as template for a polymerase chain reaction–based assay testing for the C282Y and H63D mutations as described previously. The frequencies of the C282Y and H63D mutations in patients with PCT were compared with a control group of 115 healthy volunteers (71 men and 44 women; mean±SD [median, range] age, 58±13 [58, 22-89] years) without any known liver or skin disease who were recruited from a general dental practice of the same geographical area.

The significance of the differences between the frequencies of C282Y and H63D mutations for patients with PCT and controls was determined by χ² analysis. Analysis of variance was applied to data on HFE genotype and serum iron markers. Statistical significance of differences was determined by the t test, and the χ² test was used to analyze associations among categorical variables. The significance level was defined as P<.05.

Heterozygosity for the C282Y (C282Y +/−) and H63D (H63D +/−) mutation or compound heterozygosity (H63D/C282Y) was significantly more frequent in patients with PCT compared with healthy controls (9 [15%] of 62 vs 3 [3%] of 115 [P=.007]; 18 [29%] of 62 vs 12 [10%] of 115 [P=.003]; and 8 [13%] of 62 vs 0 of 115 [P=.001], respectively) (Table 1). Whereas homozygosity for the C282Y mutation (C282Y+/+) was detected in 3 patients with PCT (3 [5%] of 62 vs 0 of 115; P=.08), homozygosity for the H63D mutation was found in neither patients nor controls (Table 1). When analyzing data on HFE genotype and serum iron markers, strikingly high values for serum iron, ferritin, and transferrin saturation were found in all 3 patients homozygous for the C282Y mutation before and after chloroquine therapy (P=.05) (Table 2). Interestingly, one of these patients did not respond to chloroquine, as characterized by persisting high urinary porphyrins and skin lesions, and the other 2 homozygous patients initially improved clinically and biochemically but relapsed within 1 year and were therefore considered as nonresponders. Remission and decrease of serum iron markers were achieved in all 3 patients after switching to phlebotomy.

Patients with PCT and wild-type HFE and those heterozygous or compound heterozygous for the C282Y or H63D mutation responded to chloroquine therapy by sustained complete remission of the skin lesions, decrease of liver enzyme activities (ALT and AST), and reduced excretion of urinary porphyrins. To further analyze the role of heterozygosity of HFE mutations, the 3 homozygotes (C282YC282Y) were excluded and the group of the remaining 35 heterozygotes were compared with the 24 patients with PCT but with wild-type HFE (Table 2 and Table 3). In the pretreatment period, patients with PCT and HFE heterozygosity (C282YWY, C282YH63D, or H63D/WY) did not have higher levels of serum iron markers than those with
wild type alone (Table 2). Baseline characteristics, urinary porphyrin excretion, liver enzymes (ALT and AST), other risk factors (alcohol and hormone intake and rates of hepatitis C virus infection), and the cumulative dose of chloroquine did not differ among these groups (Table 3). In the group of 24 patients with wildtype HFE genes, intraindividual comparison before and after treatment showed a highly significant decrease of urinary porphyrin excretion and liver enzymes (ALT and AST) and a slight but significant decrease of serum iron, ferritin, and transferrin saturation (Table 2). In contrast, among the 34 heterozygous patients with PCT (C282Y/WT, C282Y/H63D, and H63D/WT), serum iron and transferrin saturation were not different before and after therapy, although a comparable decrease of urinary porphyrin excretion and liver enzymes (ALT and AST) was observed (Table 2).

Chloroquine therapy has been shown to be as safe and effective as, but more convenient than, phlebotomy in the treatment of patients with overt PCT.22-24 Phlebotomy is more invasive and time-consuming and can be accompanied by hemodynamic reactions. At present, the decision on whether to use chloroquine or phlebotomy for PCT seems to be more empirical than evidence based. A follow-up and reevaluation of chloroquine-
treated patients with PCT according to different HFE genotypes has not been reported. Hemochromatosis genotyping can help to further classify patients with PCT and associated hemochromatosis. Significant clinical consequences arise for C282Y homozygotes and, less frequently, compound heterozygotes, since diagnosing of hemochromatosis per se implicates risks of iron-related multiorgan damage (bronze diabetes), and lifelong observation is required. Elevated serum iron markers point toward an association with hemochromatosis, which is found in 2% to 27% of patients with PCT.2,11,12,16-18 In the present study we found 3 (5%) of 62 patients with PCT and hemochromatosis as defined by high serum iron markers and homozygosity for the C282Y mutation. Our data show that chloroquine therapy did not affect the markedly elevated serum iron markers in these patients, suggesting that these patients should be treated with phlebotomy to normalize the disturbed porphyrin metabolism and accumulation of toxic iron.

Chloroquine therapy was accompanied by clinical remission, improved liver enzyme activities, and markedly reduced urinary porphyrin excretion in the 24 patients (39%) with HFE wild type as well as in 35 HFE heterozygous patients with PCT (56%). Interestingly, treatment with the drug decreased serum iron markers in the former but not in the latter group (Table 2). Other factors such as alcohol consumption, ingestion of estrogens, chronic hepatitis C, and the cumulative dose of chloroquine did not correlate with the observed differences (Table 3). In accordance with our observations in PCT patients with HFE wild type, chloroquine significantly reduced serum iron markers and liver iron accumulation in normal and iron overloaded rats.20 The weak base chloroquine elevates the pH in acidic cellular organelles and impedes the release of iron from the transferrin–transferrin receptor complex. Mutations of the HFE protein appear to modulate the function of the transferrin–transferrin receptor in favor of intracellular iron deposition, a process that is possibly opposed by chloroquine.

In the present study, as well as in reports from the United Kingdom, the United States, and Australia, the proportion of heterozygosity for C282Y and compound heterozygosity (C282Y/H63D) was significantly increased in patients with PCT.12,16 Both of these genotypes, and in particular compound heterozygosity (C282Y/H63D), which were significantly more frequent in our patients with PCT (Table 1), were described to increase the risk for iron accumulation and development of clinical hemochromatosis.20,31 Recently, an interesting model was established in which complete as well as incomplete (heterozygous) disruption of the HFE gene caused significant hepatic uroporphyrin accumulation in mice.32 This supports a direct role of heterozygosity for the C282Y mutation in the pathogenesis of PCT.

Furthermore, in our study the overall frequency of the H63D mutation and the genotype H63D/WT were increased significantly compared with controls (20% vs 5.2% and 29% vs 10%, respectively) (Table 1). Heterozygous carriers (H63D/WT) of the H63D mutation were considered to have only a slightly increased risk for iron accumulation.27 Alternatively, the H63D mutation could be associated with hidden mutations that otherwise contribute to the manifestation of PCT.

Because liver biopsy procedures were not performed or because formerly stored liver biopsy specimens of these patients were not available, we were not able to compare hepatic iron content before and after therapy. Furthermore, we could not check for URO-D deficiency (familial PCT) because of lack of access to methodology in the former East Germany. From other studies it is known that one third to one half of patients with PCT in Germany have the familial variant.33 Previously, it was reported that hereditary URO-D deficiency did not play a role in modulating demographic or clinical features of PCT.34 Therefore, it seems unlikely that URO-D deficiency would have affected our results.

We can clearly derive the following conclusions from our investigation: (1) There is a high prevalence of the C282Y and H63D mutations of HFE in patients with PCT from Saxony. (2) Simple or compound heterozygosity of HFE mutations did not affect the therapeutic response to chloroquine in PCT. (3) Because HFE homozygotes did not respond to chloroquine and decrease of serum iron markers was limited to patients with PCT and HFE wild type, phlebotomy should be first-line therapy in patients with PCT and HFE mutations.

Accepted for publication September 5, 2002.

We thank Herbert Bonkovsky, MD, for valuable critical comments. We wish to thank Claudia Hanel for excellent technical assistance and Andreas Eichler, MD, for providing blood samples from healthy volunteers.

Corresponding author and reprints: Ulrich Stolzel, MD, Medical Physics, Medizinische Klinik II, Klinikum Chemnitz gGmbH, Teaching Hospital of the University of Leipzig, Flemmingstr 2, D-09116 Chemnitz, Germany (e-mail: u.stoelzel@skc.de).

REFERENCES

10. Doss M. Hepatic porphyrins: pathobiocchemical, diagnostic, and therapeutic im...


News and Notes

These upcoming meetings are sponsored by the International Society of Dermatology:

The meeting for Therapeutic Innovation in Dermatology and Dermato-Cosmetology will take place from July 13 through July 16, 2003, in Bangkok, Thailand. For more information, telephone Dr Thada Piampongsant at 662 246-1280 (fax: 662 443-7923; e-mail: ThadaPing@Thaicosderm.org).

The Ninth International Congress on Dermatology will take place from May 19 through May 22, 2004, in Beijing, China. For more information, telephone the International Congress Secretariat at 86-10-652-891x1606 (fax: 86-10-651-237-54; e-mail: ICD2004@Chinamed.com.cn).