Natural history of C282Y homozygotes for hemochromatosis

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PURPOSE: To study the clinical outcomes of subjects who are homozygous for the C282Y mutation of the hemochromatosis gene.

SUBJECTS AND METHODS: All patients referred to a tertiary referral centre for hemochromatosis were included. The study also included 16 C282Y homozygotes detected in a population screening study.

RESULTS: The study comprised 277 C282Y homozygotes, including 16 nonexpressing C282Y homozygotes. The mean follow-up period was 7.3 years (range zero to 44 years). The actuarial survival rates of C282Y homozygotes at five, 10 and 20 years were 95%, 93% and 66%, respectively. Life-threatening diseases (cirrhosis, hepatocellular carcinoma, diabetes, heart disease) were present in 36% of male C282Y homozygotes and 19% of female C282Y homozygotes. Cirrhosis of the liver and diabetes were the major clinical symptoms affecting long term survival. Only one nonexpressing homozygote required venesection therapy during the follow-up period.

CONCLUSIONS: Long term survival is excellent in C282Y homozygotes diagnosed and treated before the development of cirrhosis and diabetes.

Key Words: Genetic testing; Hemochromatosis; HFE gene; Iron
The diagnosis of hemochromatosis has previously been based on a combination of clinical and biochemical parameters, such as typical signs and symptoms, transferrin saturation, serum ferritin concentration, liver biopsy, hepatic iron concentration and amount of iron removed by venesection therapy (1). Pedigree studies demonstrating another iron-loaded family member have provided the strongest evidence for hereditary hemochromatosis (2,3). Since the discovery of the hemochromatosis gene (HFE) in 1996 (4), many studies have demonstrated that more than 90% of typical patients with hemochromatosis are homozygous for the C282Y mutation of the HFE gene (2,3,5-8). Genetic testing has also uncovered subjects who are homozygous for the C282Y mutation but do not express iron overload.

The natural history of hemochromatosis has been studied in patients referred for evaluation and their families. Iron depletion by phlebotomy has been instituted, and the long term follow-up has been studied. Previous studies were completed before the discovery of the hemochromatosis gene and, therefore, likely included patients with alcoholic siderosis or iron overload secondary to cirrhosis of other causes. Cirrhosis and diabetes have been the major factors affecting long term survival. We describe the factors affecting the long term survival of a cohort of patients defined by genetic testing. Unlike other follow-up studies, this study included C282Y homozygotes without iron overload.

PATIENTS AND METHODS

Patient population

Patients were drawn from clinic records at a tertiary care centre specializing in hemochromatosis (n=261), as well as from a population screening study of blood donors (n=16). Before the use of genetic testing, hemochromatosis was diagnosed in proband cases based on the clinical history, physical examination, transferrin saturation, serum ferritin concentrations and confirmatory liver biopsy. Stored blood samples made genetic testing possible many years after the original phenotypic diagnoses were made. Pedigree studies were undertaken in all families. All patients had European ancestry. Discovered cases were family members who were also found to be homozygous, originally by human leukocyte antigen (HLA) typing and subsequently by C282Y genotyping. A nonexpressing homozygote was considered to be a C282Y homozygote with a transferrin saturation of less than 55% and a ferritin concentration of less than 300 µg/L. Iron-loaded patients were treated with weekly 500 mL venesections until the serum ferritin concentration was approximately 50 µg/L. Patients with a rising serum ferritin concentration after iron depletion were treated with maintenance venesection therapy three to four times per year. There were five patients with concomitant alcohol abuse (more than 60 g/day) and one patient with hepatitis C.

Comorbidities: Liver biopsies were classified as cirrhotic or noncirrhotic without knowledge of the clinical outcome of the patient. Cirrhosis was also determined at the time of autopsy in three cases. Patients were considered to be diabetic if they required insulin or oral hypoglycemic therapy. The presence of arthritis was established by history and physical examination. Heart disease was defined as symptomatic congestive heart failure (confirmed by clinical examination, chest x-ray, electrocardiogram and echocardiogram) and/or life-threatening arrhythmias. Follow-up status was determined by telephone interview with the patient and/or primary care physician.

Genetic testing

Genetic testing for the C282Y mutation was done by restriction enzyme gel digestion polymerase chain reaction using sense primer 5’TGGCAAGGGTAAACAGATCC...
and antisense primer 5'TACCTCCTCAGGCACTCCTC. These primers were used to avoid the potential mistyping in the presence of a 5569A polymorphism that has been described (9,10).

Statistical analysis
Differences in survival between groups were assessed with the Kaplan-Meier life-table method (Winstat, USA). The survival curve in the 277 C282Y homozygotes was compared with their expected survival curve derived from provincial life-table data matched for age and sex (11) (Figure 1).

The risk factors affecting survival were studied using sequential, backward stepwise proportional hazards regression (SAS software, SAS Institute, USA).

RESULTS
The demographic, clinical and biochemical profile of the C282Y homozygotes is shown in Table 1.

Nonexpressing homozygotes
There were 16 nonexpressing C282Y homozygotes. Seven of these people were detected in a population screening study of 5211 blood donors (12), and eight were found through pedigree studies. One person volunteered to be a control patient in a hemochromatosis study. The nonexpressing homozygotes included three males and 13 females (mean age 41 years, range 10 to 72 years). The mean transferrin saturation was 36% (range 16% to 53%), and the mean ferritin concentration was 108 µg/L (range 9 to 268 µg/L). Lowering the threshold for inclusion to a transferrin saturation of less than 45% and a ferritin concentration of less than 200 µg/L in women would have reduced the sample size to 11 people. The average follow-up period was 3.2 years (0.3 to 22 years). Dietary history was not informative. A 63-year-old man’s ferritin concentration rose from 67 to 405 µg/L over a three-year period, and he underwent five venesections. No other patient developed abnormal iron studies requiring venesections. One woman aged 71 years died suddenly two months after hemochromatosis was diagnosed, presumably from a cardiac cause, in her sleep. All other patients remained asymptomatic.

TABLE 1
Clinical and biochemical profile of 277 subjects who are homozygous for the C282Y mutation of the hemochromatosis gene

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Male probands (n=107)</th>
<th>Male discovered (n=64)</th>
<th>Male screened (n=5)</th>
<th>Female proband (n=44)</th>
<th>Female discovered (n=46)</th>
<th>Female screened (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, years (range)</td>
<td>50 (18-74)</td>
<td>48 (10-72)</td>
<td>43 (28-53)</td>
<td>48 (18-77)</td>
<td>50 (11-77)</td>
<td>40 (28-57)</td>
</tr>
<tr>
<td>Ferritin, µg/L (range)</td>
<td>1919 (12-8002)</td>
<td>1022 (10-5844)</td>
<td>383 (89-871)</td>
<td>684 (11-3630)</td>
<td>462 (9-2310)</td>
<td>47 (8-143)</td>
</tr>
<tr>
<td>Transferrin saturation, % (range)</td>
<td>80 (20-100)</td>
<td>71 (23-100)</td>
<td>55 (47-79)</td>
<td>61 (17-100)</td>
<td>66 (15-100)</td>
<td>45 (13-67)</td>
</tr>
<tr>
<td>Hepatic iron concentration, µmol/g (range)</td>
<td>301 (4-772)</td>
<td>226 (28-772)</td>
<td>No biopsies</td>
<td>153 (20-322)</td>
<td>182 (36-482)</td>
<td>No biopsies</td>
</tr>
<tr>
<td>Cirrhosis, n (%)</td>
<td>31 (30)</td>
<td>4 (6)</td>
<td>0</td>
<td>3 (7)</td>
<td>2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Arthritis, n (%)</td>
<td>46 (43)</td>
<td>16 (25)</td>
<td>0</td>
<td>14 (32)</td>
<td>10 (22)</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>22 (21)</td>
<td>5 (8)</td>
<td>0</td>
<td>5 (11)</td>
<td>6 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Congestive heart failure, n (%)</td>
<td>13 (12)</td>
<td>6 (9)</td>
<td>0</td>
<td>2 (5)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Life-threatening symptoms*, n (%)</td>
<td>49 (46)</td>
<td>14 (22)</td>
<td>0</td>
<td>8 (18)</td>
<td>11 (24)</td>
<td>0</td>
</tr>
</tbody>
</table>

Reference ranges: serum ferritin concentration 15 to 200 µg/L for women and 30 to 300 µg/L for men; transferrin saturation 20% to 55%; hepatic iron concentration 0 to 35 µmol/g. *Cirrhosis, hepatocellular carcinoma, diabetes or heart disease (many patients had more than one of these symptoms).

TABLE 2
Summary of model results of sequential, backward stepwise proportional hazards regressions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risk ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>3.95</td>
<td>1.6-10.0</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>2.92</td>
<td>1.2-7.5</td>
</tr>
<tr>
<td>Diagnosis after age 50 years</td>
<td>2.68</td>
<td>1.7-4.3</td>
</tr>
<tr>
<td>Arthritis*</td>
<td>0.29</td>
<td>0.1-0.8</td>
</tr>
</tbody>
</table>

*The apparent protective effect of arthritis is the reciprocal of the risk of not having arthritis, eg, 1/0.29=3.4

Pression 5'TACCTCCTCAGGCACTCCTC. These primers were used to avoid the potential mistyping in the presence of a 5569A polymorphism that has been described (9,10).
Cause of death
During a mean follow-up of 7.3 years (range zero to 44 years), there were 24 deaths. In nine of these cases, the patients were inferred to be homozygous for the C282Y mutation based on pedigree analysis, HLA typing or the presence of a living relative who was homozygous for the C282Y mutation. The causes of death that were considered to be directly related to hemochromatosis were hepatocellular carcinoma (n=9), congestive heart failure (n=2), arrhythmia (n=1), complications of diabetes (n=2) and septicemia following liver transplantation (n=1). There was one case of hepatocellular carcinoma diagnosed without concomitant cirrhosis. The other causes of death were myocardial infarction (n=4), pneumonia (n=1), and cancer of the lung (n=1), pancreas (n=1), brain (n=1) and breast (n=1).

Prevalence of life-threatening complications
The prevalence of cirrhosis, hepatocellular carcinoma, congestive heart failure and diabetes in treated probands and discovered family members is shown in Table 1. Cirrhosis was less frequent in discovered cases than in proband cases; however, liver biopsies were performed less frequently in discovered cases. The prevalence of cirrhosis may be underestimated in this study because not all patients had liver biopsies. None of the 16 patients found through population screening had life-threatening symptoms. Life-threatening symptoms were more common in proband cases and were present in 36% of men and 19% of women (Table 1).

Cumulative survival
The cumulative survival rates of the entire group of C282Y homozygotes were 95% at five years, 93% at 10 years and 66% at 20 years. The rate of survival was lower in diabetic than in nondiabetic patients, and lower in cirrhotic than in noncirrhotic patients (P<0.05, log rank test) (Figure 1). The major factors affecting survival according to multivariable analysis were diabetes and cirrhosis (Table 2).

DISCUSSION
In the present study, 277 C282Y homozygotes were followed for up to 44 years. This study differed from previous survival studies of patients with hemochromatosis, in that only C282Y homozygotes were included (13-17). The study also included nonexpressing C282Y homozygotes without evidence of iron overload. As a result of this selection of patients, the cumulative survival rate was better than that reported in 1991, in a study of 88 patients with hemochromatosis (13). This increased survival rate is likely the result of earlier diagnosis, before the development of cirrhosis and diabetes. However, survival may have been overestimated in the present study because patients had to have been alive in 1996 to undergo genetic testing for hemochromatosis. Genetic status was inferred in eight deceased patients, where possible, with pedigree analysis and HLA typing. This study has demonstrated that diabetes and cirrhosis are
Natural history of hemochromatosis

We have not described the clinical features of iron overload in patients with idiopathic iron overload without HFE mutations. At the London Health Science Centre, this represents only 3% of cases, which are all are isolated, nonfamilial cases that may represent a heterogeneous collection of etiologies, with iron overload as a common feature. As a result, the effectiveness of genetic counselling is speculative in this patient population. The proportion of people with non-HFE-related iron overload varies geographically, and this disorder has been most commonly described in Italians and Africans.

SUMMARY

The prognosis of a young C282Y homozygote diagnosed before the development of cirrhosis or diabetes is excellent because the rate of survival does not differ from that of the general population. Unfortunately, the presence of symptoms is often associated with organ damage, which underscores the importance of early detection and prompt treatment. Large scale population screening projects are in progress in North America, Europe and Australia, which should resolve many of the controversies surrounding screening for hemochromatosis (33).

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REFERENCES


