ORIGINAL CONTRIBUTIONS

Hereditary Hemochromatosis: Effect of Excessive Alcohol Consumption on Disease Expression in Patients Homozygous for the C282Y Mutation

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Hereditary hemochromatosis is a common inherited disorder characterized by iron overload. A single mutation (C282Y) in the HFE gene is present in 80–95% of cases in populations of northern European extraction. The disorder presents a large phenotypic heterogeneity, and its expression can be influenced by environmental factors. This 1977–2002 study aimed to identify the influence of alcohol consumption on expression of the disease. The authors retrospectively registered 378 C282Y-homozygous patients treated in a blood center of western Brittany, France. In this cohort, 33 patients reported excessive alcohol consumption (8.7%). Those subjects presented significantly increased iron parameters (serum ferritin: 1,745.2 vs. 968.7 µg/liter, p < 0.0001; serum iron: 39.9 vs. 36.0 µmol/liter, p = 0.0040; transferrin saturation: 87.1 vs. 80.1%, p = 0.0071) and elevated liver enzymes (alanine aminotransferase: 66.3 vs. 41.1 IU/liter, p = 0.0003; aspartate aminotransferase: 56.2 vs. 34.9 IU/liter, p = 0.0002). Their risk of skin pigmentation was also higher (odds ratio = 3.4, p = 0.0006). Results remained unchanged after adjustment. This study provides precise quantitative data about the impact of alcohol on expression of hereditary hemochromatosis in C282Y-homozygous patients. Excessive alcohol consumption accentuates disease expression and therefore the risk of cirrhosis and cancer. Consequently, these patients should be encouraged to consume very moderate quantities of alcohol.

alcohol drinking; disease susceptibility; hemochromatosis; hereditary diseases; iron overload

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; OR, odds ratio.

Hereditary hemochromatosis is a common genetic disease of autosomal recessive inheritance that occurs predominantly in populations of northern and western European descent (prevalence: 1–4/1,000). It is characterized by excessive digestive absorption of iron, leading to its progressive accumulation in different tissues of the body (notably liver, pancreas, and heart) and then to alteration of the structure and function of these organs. The natural history of hereditary hemochromatosis includes three stages: latency, biologic expression (appearing rarely before the age of 20 years and corresponding to increased iron parameters, such as serum iron, transferrin saturation, and serum ferritin), and clinical expression. The first clinical symptoms appear generally at about age 40 years in males and later in females, approximately age 50 years, because of the protective effects of menstrual blood loss and of pregnancies. These clinical symptoms are nonspecific and include fatigue, skin pigmentation, hepatomegaly, arthritis, diabetes, and cardiomyopathy (1, 2).

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Hereditary hemochromatosis is one of the sole genetic
diseases benefiting from simple and efficient treatment when
implemented early. Treatment relies on regular therapeutic
venesection, generally weekly, until iron depletion occurs
(i.e., normalization of iron parameters) and is followed by a
maintenance treatment. Without early implementation of
such a treatment, the disease has a poor prognosis and there-
fore can evolve toward irreversible damage, such as hepato-
cellular carcinoma or heart failure (1–3).

In 1996, a candidate gene for hereditary hemochromatosis
was cloned on chromosome 6, at position 6p21.3 (4). This
gene, HFE, encodes the HFE protein, which is a transmem-
brane glycoprotein implied in modulation of iron uptake (4,
5). The gene contains a main mutation, C282Y, corre-
sponding to substitution of a tyrosine for a cysteine at amino
acid 282 and preventing formation of a disulfide bond (4, 6).
This mutation, whose allelic frequency varies between 0.5
and 10 percent in Caucasian populations (7, 8), is present in
a homozygous state in 80–95 percent of patients from
northern Europe (4, 7, 9–11). In some US populations, only
60 percent of hemochromatosis patients are homozygous for
this mutation (12). Besides this C282Y mutation, two other
susceptibility factors associated with hered-
itary hemochromatosis (H63D, S65C) and about 10 rare
mutations have been identified in the HFE gene (9, 13–16).

Discovery of the HFE gene has enabled a better under-
standing of the physiopathologic mechanisms implied in
hereditary hemochromatosis. However, this pathology
remains complex and presents a large phenotypic heteroge-

ty (17–19). The different mutations identified in the HFE
gene do not have the same penetrance, and rapidity of the
evolution of iron overload can be modified by factors that
may reduce the iron stores (blood donation) or increase them
(intake of iron). Moreover, the severity of the disease can
vary in patients with similar degrees of iron overload. For
example, the risk of cirrhosis is increased by excessive
alcohol consumption or the presence of viral hepatitis (20),
while the risk of cardiomyopathy is increased by high intake
of vitamin C, which potentiates iron uptake. Phenotypic
expression of hereditary hemochromatosis can therefore be
influenced by environmental factors and is the result of inter-
actions between the gene and modifying factors.

The aim of the present study (1977–2002) was to identify
the influence of excessive alcohol consumption on expres-
sion of the disease in patients homozygous for the main
mutation (C282Y). To do so, we analyzed a cohort of 378
hemochromatosis subjects treated in a blood center of
western Brittany, France, where the frequency of the disease
is particularly high (21). This study showed that excessive
alcohol consumption significantly increases the phenotypic
expression of hereditary hemochromatosis.

MATERIALS AND METHODS

Study population

Brittany is a region of approximately 3 million inhabitants
located in the western part of France. Its population is rela-
tively ethnically homogenous; most of the inhabitants are of
Celtic origin. This study included 378 C282Y-homozygous
hemochromatosis patients treated in a blood center of
western Brittany whose transferrin saturation concentration
was greater than or equal to 45 percent and for whom the
questionnaire item on alcohol consumption was documented
correctly.

Clinical questionnaire

At a patient’s first visit to the blood center, a physician
specialist completed a clinical questionnaire. It contained
information on sociodemographic characteristics of the
patient (e.g., gender, age, age at disease onset, circumstances
of disease diagnosis, lifestyle factors, height, weight), clin-
ical signs (fatigue, skin pigmentation, hepatomegaly,
arthritis, cardiomyopathy, metabolic disorders (i.e., diabetes,
hypercholesterolemia, hypertriglyceridemia), and biochem-
ical parameters (serum ferritin, serum iron, transferrin satu-
ration, alanine aminotransferase (ALT), aspartate
aminotransferase (AST)). Also available were data on the
treatment of patients, such as number and quantity of venu-
sections and quantity of iron removed. This questionnaire
also included a detailed item on daily consumption of
alcohol, measured by the number of glasses of alcohol drunk
each day (including wine, beer, and liquors). These data
eabled the quantity of ethanol (in grams) consumed each
day, by each patient in the cohort, to be determined. Exces-
sive alcohol consumption was defined as daily consumption
of more than or equal to 60 g. Very moderate alcohol
consumption was defined as less than 20 g of ethanol per
day.

Measurement of iron parameters and determination of
HFE genotype

Serum iron concentrations were measured by using stan-
dard biochemical methods. The normal range of serum
ferritin for women was 15–200 µg/liter and for men was 30–
300 µg/liter. The normal range of transferrin saturation was
20–45 percent. DNA samples harboring the C282Y mutation
were identified by polymerase chain reaction and restriction
enzyme assays, as described previously (13, 21), and, more
recently, by denaturing high-performance liquid chromatog-
raphy (22).

Statistical analysis

In this paper, quantitative variables are expressed as mean
(standard deviation). Differences in means between groups
were tested by using Student’s t test or the Mann-Whitney U

test. Qualitative values are presented as percentages and
were compared by using the chi-square test or Fisher’s exact
test in case of sample size samples. Because the distribution
of the serum ferritin variable was highly skewed, logarithmic
transformation was performed to normalize the measures for
all statistical analyses. A p value of less than 5 percent was
considered significant. Statistical analyses were conducted
by using Epi-Info software (version 6.04; Centers for
Disease Control and Prevention, Atlanta, Georgia).

The biochemical and clinical characteristics of the whole

cohort were described. Then, the biologic characteristics of
patients according to their alcohol consumption (≥60 vs. <60 g/day) were compared. The influence of this factor on disease expression, measured by levels of serum ferritin, serum iron, and transferrin saturation, was assessed by using a linear regression analysis. In a first step, we conducted a univariate analysis and then a multivariate analysis with adjustment for potential confounding factors such as gender and age. Moreover, the frequency of clinical signs observed in patients reporting excessive alcohol consumption was compared with that observed in patients reporting lower alcohol consumption. Odds ratios with 95 percent confidence intervals were calculated.

This study fulfilled the bioethical rules in place in France. Informed consent was obtained from patients before blood samples were taken.

RESULTS

Study population

This cohort included 378 subjects, of whom 60.3 percent were male (sex ratio: 1.5). The age of patients ranged from 18 to 80 years. Age at onset tended to be higher in females than in males (48.8 vs. 46.5 years, p = 0.053). The diagnosis was made mainly on the basis of clinical features (57.4 percent), less frequently through family testing (30.7 percent) or during an occupational medicine visit (9.5 percent). Of those patients, 8.7 percent reported excessive alcohol consumption (≥60 g/day, n = 33). This group included mainly males (n = 31): 13.6 percent of them estimated their alcohol consumption to be equal to or greater than 60 g per day (31/228) compared with only 1.3 percent of females (2/150) (p < 0.0001). Mean number of glasses of alcohol consumed each day was significantly higher for males than for females (2.2 vs. 0.4; p < 10^{-6}), the overall range being 0–13 glasses per day. Mean age at onset was not significantly lower in men reporting excessive alcohol consumption compared with those consuming smaller quantities (45.8 vs. 46.6 years, p = 0.71). For women, this analysis was difficult because the number of women in the “excessive” group was low (n = 2).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gender*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Sociodemographic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>228</td>
<td>150</td>
</tr>
<tr>
<td>Age at disease onset (years)</td>
<td>46.5 (12.1)</td>
<td>48.8 (14.2)</td>
</tr>
<tr>
<td>Biochemical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ferritin (µg/liter)</td>
<td>1,388.0 (1,329.8)</td>
<td>522.3 (785.3)</td>
</tr>
<tr>
<td>Serum iron (µmol/liter)</td>
<td>37.7 (7.1)</td>
<td>34.4 (7.5)</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>82.7 (13.4)</td>
<td>77.9 (13.2)</td>
</tr>
</tbody>
</table>

* All values except those for no. of patients are expressed as mean (standard deviation).

Characteristics of patients according to alcohol consumption

Frequency of excessive alcohol consumption. In this cohort, 14.8 percent of the hereditary hemochromatosis patients declared that they were nondrinkers, 50.3 percent that they consumed alcohol occasionally, and 34.9 percent that they consumed alcohol regularly. In all, 8.7 percent of the hereditary hemochromatosis patients in this cohort reported excessive alcohol consumption (n = 33). This group included mainly males (n = 31): 13.6 percent of them estimated their alcohol consumption to be equal to or greater than 60 g per day (31/228) compared with only 1.3 percent of females (2/150) (p < 0.0001). Mean number of glasses of alcohol consumed each day was significantly higher for males than for females (2.2 vs. 0.4; p < 10^{-6}), the overall range being 0–13 glasses per day. Mean age at onset was not significantly lower in men reporting excessive alcohol consumption compared with those consuming smaller quantities (45.8 vs. 46.6 years, p = 0.71). For women, this analysis was difficult because the number of women in the “excessive” group was low (n = 2).

FIGURE 1. Frequency of the most common clinical signs of hereditary hemochromatosis, according to gender, observed in 378 C282Y-homozygous patients in western Brittany (France), 1977–2002.
Biochemical parameters according to alcohol consumption. Results of the comparison of biochemical data between hereditary hemochromatosis patients who did and those who did not consume excessive quantities of alcohol are presented in table 2. Iron parameters were significantly increased in patients who drank at least 60 g of alcohol per day (serum ferritin: 1,745.2 vs. 968.7 \( \mu g/liter \), \( p < 0.0001 \); serum iron: 39.9 vs. 36.0 \( \mu mol/liter \), \( p = 0.0040 \); transferrin saturation: 87.1 vs. 80.1 percent, \( p = 0.0071 \)). Table 2 also reports the results of the linear regression analyzing the relation between alcohol consumption and iron overload, measured by the logarithm of serum ferritin, serum iron, and transferrin saturation. These results remained unchanged after adjustment for gender and age. Moreover, ALT and AST levels were also significantly higher in subjects reporting heavy alcohol consumption (table 2). These data were available for only 265 and 262 patients, respectively. Mean of ALT was 66.3 IU/liter (standard deviation, 48.1) in patients declaring heavy alcohol consumption versus 41.1 IU/liter (standard deviation, 28.3) in those whose level of alcohol consumption was lower (\( p = 0.0003 \)). Similarly, mean of AST was 56.2 IU/liter (standard deviation, 47.8) in the group of patients reporting excessive alcohol consumption versus 34.9 IU/liter (standard deviation, 18.4) in the other group (\( p = 0.0002 \)). Patients who did not undergo ALT and AST tests corresponded to patients who had begun their treatment more than 10 years ago, before these examinations began to be conducted. The patients who had had ALT and AST tests were not significantly different from those who did not have these tests.

Clinical signs according to alcohol consumption. The frequency of major clinical signs of hereditary hemochromatosis observed in patients declaring excessive alcohol consumption compared with signs observed in patients reporting lower alcohol consumption is shown in figure 2. Patients who drank more than 60 g of alcohol per day had a significantly increased risk of skin pigmentation (51.5 vs. 24.0 percent; OR = 3.4, 95 percent CI: 1.5, 7.4; \( p = 0.0006 \)). They also tended to have a higher risk of diabetes (26.3 vs. 11.1 percent; OR = 2.9, 95 percent CI: 0.8, 9.9; \( p = 0.058 \)) and hepatomegaly (25.9 vs. 14.3 percent; OR = 2.1, 95 percent CI: 0.8, 5.7; \( p = 0.108 \)). A history of viral hepatitis was observed in two of the patients who drank at least 60 g of alcohol per day. The biologic and clinical data for these two patients did not significantly differ from those for the 31 other patients.

**DISCUSSION**

The present study highlights the phenotypic expression of hereditary hemochromatosis in patients homozygous for the main mutation of the \( HFE \) gene (\( C282Y \)) and provides precise quantitative data about the impact of alcohol consumption on expression of the disease in those patients. This study shows that excessive alcohol consumption increases the severity of the disease in \( C282Y \)-homozygous

![FIGURE 2. Frequency of the most common clinical signs of hereditary hemochromatosis, according to alcohol consumption (≥60 or <60 g per day), observed in 378 \( C282Y \)-homozygous patients in western Brittany (France), 1977–2002.](image-url)
patients, resulting in higher iron parameters and more frequent clinical signs. Alcohol consumption associated with a genetic factor increases expression of the disease and therefore the risk of cirrhosis and cancer. Excessive alcohol consumption is thus an aggravating factor.

In our study, the prevalence of excessive alcohol consumption among hereditary hemochromatosis patients (8.7 percent) was lower than that reported in previous studies, which were performed before the \textit{HFE} gene was discovered and which should therefore have included, as hereditary hemochromatosis patients, subjects who had alcoholic siderosis \cite{23, 24}. For example, in a study performed in 1992, Loreal et al. \cite{23} showed that in their cohort of 127 patients, 29 percent were alcoholic. Before the genetic test existed, the distinction between patients affected with hereditary hemochromatosis and those affected with alcoholic siderosis was difficult to establish. Therefore, most studies should have considered some patients with alcoholic siderosis as having hereditary hemochromatosis. Discovery of the \textit{HFE} gene in 1996 has provided a complementary element for helping to set the diagnosis of hereditary hemochromatosis. Our study measured the effect of alcohol consumption in hereditary hemochromatosis patients whose diagnosis was not ambiguous and who were homozygous for the main mutation of the \textit{HFE} gene. In 1995, Adams and Agnew \cite{25} showed similar results. They focused their study on diagnosis criteria based on the presence of a human-lymphocyte-antigen identical sibling with iron overload. Heavy alcohol consumption (>80 g/day) was observed in 15 percent of their hemochromatosis patients \cite{25}.

Note that declaration of alcohol consumption is subjective \cite{26, 27} and that consumption must certainly be, at least in some cases, underestimated. However, this issue is addressed in all studies dealing with alcohol consumption and should not have affected our study more than others.

In this study, we showed that excessive alcohol consumption increased the severity of the disease, characterized by more frequent clinical signs, notably skin pigmentation, diabetes, and hepatomegaly. It is known that alcohol worsens the hepatic damage produced by iron in hereditary hemochromatosis. Several studies reported that excessive alcohol consumption greatly increased the prevalence of hepatic fibrosis and cirrhosis in hereditary hemochromatosis patients \cite{23, 28}. For example, in a recent study, Fletcher et al. \cite{28} quantified the contribution of excessive alcohol consumption to the development of cirrhosis in a cohort of 224 \textit{C282Y}-homozygous hemochromatosis subjects. These authors clearly observed that patients who reported excessive alcohol consumption (>60 g per day) seemed nine times more likely to develop cirrhosis compared with subjects who drank less than this quantity. The frequency of cirrhosis reached 61.1 percent in the group of heavy drinkers, whereas it was uncommon in the other group of patients (7.1 percent). Moreover, the authors showed that patients reporting heavy alcohol consumption were more likely to develop cirrhosis at an earlier age. Indeed, the mean age of patients who consumed excessive quantities of alcohol was 46.5 years (standard deviation, 10.5) compared with 53.7 years (standard deviation, 11.9) in patients who drank less than 60 g of alcohol per day. Fletcher et al. also reported that the findings were not significantly modified by considering a threshold, for alcohol consumption, of 40 g per day.

In our study, the influence of alcohol consumption on the risk of extensive liver fibrosis or cirrhosis could not be estimated. A liver biopsy was performed for the majority of the patients, but the population sample was not sufficient to enable us to draw conclusions.

Iron overload itself can result in hepatic fibrosis and cirrhosis. However, in hemochromatosis patients, this overload should be influenced by cofactors such as hepatitis or alcohol \cite{20, 23–25}. According to Adams and Agnew, “patients with heavy alcohol consumption had a higher prevalence of cirrhosis at the time of diagnosis without a significant increase in iron overload, suggesting an additive hepatotoxic effect of the alcohol rather than any secondary effects on iron absorption or metabolism” \cite[p. 726]{25}. Chronic alcohol consumption therefore has an additive hepatotoxic effect. In a recent editorial, Britton and Bacon \cite{29} explained the possible mechanisms by which the combination of alcohol and iron overload lead to hepatic fibrogenesis. This occurrence may be the result of two phenomena: first, both iron overload and alcohol induce oxidative stress in the liver, causing oxidative injury and fibrogenesis; second, these two factors may also produce hepatocellular damage by nonoxidative mechanisms \cite{29}.

Our findings have implications for the public health field in terms of the adoption of preventive strategies. Alcohol consumption associated with genetic factors increases the severity of hereditary hemochromatosis and therefore the risk of cirrhosis and cancer. Consequently, patients who have the disease should be discouraged from consuming excessive quantities of alcohol because of the added hepatotoxicity it induces. In any case, treatment by regular venesection is recommended to reduce iron stores to normal values and to decrease the risk that the disease will progress, causing irreversible damage \cite{28, 29}.

In conclusion, phenotypic expression of hereditary hemochromatosis is influenced by exogenous risk factors such as alcohol consumption, as illustrated in this study. The severity of the disease is the result of interactions between genetic and environmental factors. Therefore, the pathology of hereditary hemochromatosis is multifactorial and, with this disease, it is interesting to analyze the complexity of gene-gene and gene-environment interactions \cite{19}.

\section*{Acknowledgments}

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\section*{References}


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