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Authors
Adams, Paul C
Speechley, Mark
Barton, James C
et al.

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Probability of C282Y homozygosity decreases as liver transaminase activities increase in participants with hyperferritinemia in the HEIRS Study

Paul C. Adams1, Mark Speechley2, James C. Barton3, Christine E. McLaren4, Gordon D. McLaren5, and John H. Eckfeldt6

1Division of Gastroenterology, Department of Medicine, London Health Sciences Centre, London, Ontario
2Department of Epidemiology & Biostatistics, University of Western Ontario, London, Ontario
3Southern Iron Disorders Center, Birmingham, Alabama and Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama
4Department of Epidemiology, University of California, Irvine, CA
5Division of Hematology/Oncology, Department of Medicine, University of California, Irvine, CA, VA Long Beach Healthcare System, Long Beach, CA
6Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota

Abstract

Background—Hemochromatosis is considered by many to be an uncommon disorder, although the prevalence of HFE C282Y homozygosity is relatively high in Caucasians. Liver disease is one of the most consistent findings in advanced iron overload due to hemochromatosis. Liver clinics are often thought to be ideal venues for diagnosis of hemochromatosis, but diagnosis rates are often low.

Methods—The Hemochromatosis and Iron Overload Screening (HEIRS) Study screened 99, 711 primary care participants in North America for iron overload using serum ferritin and transferrin saturation measurements and HFE genotyping. In this HEIRS substudy, serum hepatic transaminases activities (ALT, AST) were compared between 162 C282Y homozygotes and 1,367 non-homozygotes with a serum ferritin > 300 μg/L in men and > 200 μg/L in women and transferrin saturation > 45 % in women and 50 % in men. The probability of being a C282Y homozygote was determined for AST and ALT ranges.

Results—Mean ALT and AST activities were significantly lower in C282Y homozygotes than non-homozygotes. The probability of being a C282Y homozygote increased as the ALT and AST activities decreased.

Conclusions—Patients with hyperferritinemia are more likely to be C282Y homozygotes if they have normal liver transaminase activities. This paradox could explain the low yields of hemochromatosis screening reported by some liver clinics.
Introduction

HFE hemochromatosis is one of the most common genetic disorders in Caucasians. Liver disease is the most prevalent, serious complication of iron overload due to hemochromatosis, and consequential cirrhosis and hepatocellular carcinoma are common causes of death.(1) Hemochromatosis is not an inflammatory liver disease. Liver biopsies from patients with hemochromatosis typically show iron overload, with or without liver fibrosis, and an absence of lymphocytes, leucocytes, and eosinophils. Serum alanine transaminase (ALT) and aspartate transaminase (AST) leak into the circulation due to necrosis of hepatocytes, and are routinely measured as markers of hepatocellular disease.

Many patients are referred to liver clinics for evaluation of elevations in serum ferritin. In such patients, it is common to measure serum transaminases. Other pertinent tests include serum transferrin saturation, and HFE genotyping. HBsAg and anti-HCV are tested in many patients with an elevated serum ALT. Most physicians assume that elevations of serum transaminase activities increase the probability that a patient has hemochromatosis because this is the case with many liver diseases. We found that the probability of HFE C282Y homozygosity decreases as the serum transaminase activities increase.

Methods

The study design and overall results of the Hemochromatosis and Iron Overload Screening (HEIRS) Study have been previously reported.(2-4) The HEIRS Study was approved by all local investigational review boards. Participants ≥25 years of age who gave informed consent were recruited from five Field Centers that serve ethnically and socio-economically diverse populations. All participants had random testing for serum transferrin saturation and serum ferritin levels (without intentional fasting), and genotyping to detect the common C282Y and H63D mutations of the HFE gene. Participants who reported a previous diagnosis of hemochromatosis or iron overload (treated or untreated) were excluded.

Post-screening clinical examinations were performed on participants with elevated transferrin saturation (>45 % women, > 50 % men) and ferritin (> 300 μg/L for men, > 200 μg/L for women), all HFE C282Y homozygotes, and control participants (matched for age, gender and race) with normal transferrin saturation and serum ferritin values but without HFE C282Y or H63D mutations. Of 2,265 participants invited for clinical examinations, there was a 75 % participation rate. Among C282Y homozygotes (n=333), the participation rate was 91 %. In this study, only participants with an elevated serum ferritin and transferrin saturation were analyzed because participants with a normal serum ferritin level were considered to have a low probability of having liver disease. In the HEIRS Study, an elevated serum ferritin was found in 88 % of male and 57 % of female C282Y homozygotes. (2) These clinical examinations included measurements of serum ALT, AST, and ferritin. Self-reported daily ethanol consumption was collected and reported as g/day.

For analysis, intervals of serum ALT and AST activities were analyzed: [0,19), [20,39), [40,59), [60,79), [80,99), and > 100 IU/L respectively. There were no homozygotes with AST or ALT above 119 IU/L. The probability of being a C282Y homozygote was calculated for each ALT and AST interval and for gender specific groups with and without an elevated AST and ALT (> 40 IU/L). The trend in probabilities was tested with a chi square test for linear trend with one degree of freedom. All analyses were performed using OpenEpi v. 2.3.1 (Atlanta, GA). A subgroup analysis was performed on only Caucasian participants. An adjusted Mantel-Haenszel chi-square test was used to determine if the overall trend remained after adjustment for gender. Pearson correlation coefficients were calculated for the relationship of ALT to ferritin.
Results

The participants included 80 female C282Y homozygotes, 82 male C282Y homozygotes, 575 female non-C282Y homozygotes and 792 male non-C282Y homozygotes. All participants in this study had an elevated ferritin and transferrin saturation. Of C282Y homozygotes, 97% were Caucasian. In the non-homozygotes, 41% were Caucasian.

Other genotypes in non-C282Y homozygous participants included wild type (no C282Y or H63D mutations) in 886, C282Y heterozygosity in 109, compound heterozygosity (C282Y/H63D) in 87, H63D homozygosity in 55, and H63D heterozygosity in 230. The profile of the participants is shown in Table 1. The investigation of the etiology of elevated ALT or AST activities in the non-C282Y homozygotes was beyond the primary scope of the HEIRS Study, although we previously reported the prevalence of viral hepatitis and the results of liver biopsies in selected HEIRS Study participants.(5)

Mean serum ALT and AST activities were significantly lower in C282Y homozygotes than in non-homozygotes (Table 1). ALT and AST activities were significantly lower in female C282Y homozygotes than in male homozygotes. Amongst the female homozygotes, an ALT < 30 was seen in 65/80, AST < 30 in 69/80. The distribution of ALT values in relationship to serum ferritin in male and female C282Y homozygotes and non-homozygotes is shown in Figures 1A and 1B. In these figures, it is demonstrated that many C282Y homozygotes have normal ALT but also that patients with an elevated ALT are unlikely to be C282Y homozygotes. The correlation between ALT and ferritin was stronger in C282Y homozygotes than in non-homozygotes which is consistent with an inflammatory cause of the hyperferritinemia in non-homozygotes.

The proportion of male C282Y homozygotes with a ALT and AST < 40 IU/L was 71% and 87% respectively. The proportion of female C282Y homozygotes with a ALT and AST < 40 IU/L was 87% and 95% respectively.

The decreasing probability of being a C282Y homozygote across groups in men and women with increasing ALT is shown in Figure 2. Similar results were determined for AST. P values for chi-square tests for trends in proportions for ALT for men was 0.036 and women was 0.00017. Mantel-Haenszel chi-square adjusted for gender was <0.0001. An unanticipated observation was that the probability of being a C282Y homozygote decreased as the serum ALT and AST increased. The results of subgroup analysis limited to Caucasians were similar.

Discussion

It is widely believed that the probability of diagnosing many liver diseases increases as serum transaminases increase. In the present study of subjects with hyperferritinemia, the probability of being a C282Y homozygote decreased with increasing ALT and AST. This probably occurs because the deposition of excessive iron alone in hepatocytes of persons with hemochromatosis is not inflammatory. “Silent” hepatic fibrosis occurs in some subjects with hemochromatosis and normal serum transaminases.(6, 7) On the other hand, some patients with hemochromatosis and HFE C282Y homozygosity have both hepatic iron overload and an inflammatory liver condition. For example, approximately 15% of C282Y homozygotes diagnosed in medical care have severe hepatic steatosis proven by liver biopsy. These patients had higher median serum ALT and ferritin levels than C282Y homozygotes without hepatic steatosis or other inflammatory liver disorder.(8)

In contrast, patients referred for evaluation of elevated serum ferritin levels usually have hyperferritinemia due to inflammatory liver disease rather than iron overload due to HFE.
hemochromatosis. In prospective analyses of subjects with chronic elevation of serum transaminases, hepatic steatosis associated with or without excessive ethanol consumption was the predominant cause of elevated serum transaminases. Hemochromatosis was rare in these case series.

In the present study, there is a potential bias wherein HEIRS Study non-C282Y homozygous participants were deliberately selected for post-screening clinical examinations because they had elevated serum transferrin saturation and ferritin measures. The present results demonstrate that these participants had higher mean serum transaminase activities than did HFE C282Y homozygotes. Another potential source of uncertainty is that elevations of ALT are intermittent or unreproducible in a majority of outwardly healthy subjects, whereas the present results are based on single measurements of serum transaminase activities in subjects selected for iron phenotypes and HFE genotypes. The C282Y homozygotes identified by screening in this study had relatively modest serum ferritin elevations for the most part and are not representative of patients diagnosed in practice. Homozygotes with heavier iron burdens and consequent hepatocellular damage may have elevated transaminases.

The present results demonstrate that participants who had C282Y homozygosity uncomplicated by a liver disorder associated with inflammation, e.g., steatosis or hepatitis C, are more likely to have normal serum transaminases and elevated serum ferritin levels. Persons with both elevated serum transaminase and elevated serum ferritin levels are less likely to be C282Y homozygotes. Thus, it is also predicted that the proportion of patients who present with both elevated serum transaminases and hyperferritinemia who are C282Y homozygotes with iron overload without concomitant inflammatory liver disease is relatively small. Our observations and prediction are consistent with the low rates of detection of HFE C282Y homozygotes observed in liver clinics because many of these homozygotes also have normal serum transaminases. In a retrospective analysis of physicians’ evaluations of 100 consecutive patients in whom mild elevations of ALT and AST were observed, evaluation to exclude hemochromatosis was not performed in 90% of subjects. Taken together, these observations suggest that some physicians are reluctant to evaluate patients for HFE hemochromatosis because they erroneously believe that this condition is typically associated with elevated serum transaminases. We conclude that all Caucasian patients with hyperferritinemia should be evaluated for HFE hemochromatosis, regardless of serum transaminases. Other tools that can aid in the detection of HFE hemochromatosis include elevated serum transferrin saturation and family history.

Acknowledgments

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Abbreviations

AST aspartate serum transaminase
ALT alanine serum transaminase
HEIRS Study Hemochromatosis and Iron Overload Screening Study
Reference List

Figure 1A.
Serum alanine transaminase (ALT) in male C282Y homozygotes (●) ($r=0.44$, $p < .0001$) and non-homozygotes ( ○)($r = 0.22$, $p < .0001$). Data displayed are excerpted from observations in participants with a serum ferritin < 1000 μg/L and ALT < 300 IU/L. The solid line represents the upper limit of the reference range (40 IU/L). The numbers above the bar represent the number of participants in each group.
Figure 1B.
Serum alanine transaminase (ALT) in female C282Y homozygotes (●)(r = 0.63, p < .0001) and non-homozygotes ( ○ )(r = 0.31, p < .0001). Data displayed are excerpted from observations in participants with a serum ferritin < 1000 μg/L and ALT < 300 IU/L. The solid line represents the upper limit of the reference range (40 IU/L). The numbers above the bar represent the number of participants in each group.
Figure 2.
The probability of being a C282Y homozygote in 6 groups according to serum alanine transaminase (ALT) in men (■) and women (□). Chi-square test for linear test for trend in proportions was 0.036 in men and 0.00017 in women.
Table 1

<table>
<thead>
<tr>
<th>Gender/Genotype</th>
<th>n</th>
<th>Age</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>Ferritin (μg/L)</th>
<th>Ethanol (g/d)</th>
<th>HBsAg+</th>
<th>Anti-HCV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female C282Y homozygotes</td>
<td>80</td>
<td>55 (52-57)</td>
<td>24 (19-30)</td>
<td>25 (19-31)</td>
<td>64 (539-742)</td>
<td>7.8 (0-16)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Female non-C282Y homozygotes</td>
<td>575</td>
<td>56 (55-57)</td>
<td>37.2 (34-40)</td>
<td>41.2 (38-45)</td>
<td>526 (477-574)</td>
<td>9.1 (5.8-12.3)</td>
<td>12</td>
<td>83</td>
</tr>
<tr>
<td>Male C282Y homozygotes</td>
<td>82</td>
<td>52 (49-55)</td>
<td>37 (31-42)</td>
<td>29 (26-33)</td>
<td>1118 (933-1303)</td>
<td>11 (5-16)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Male non-C282Y homozygotes</td>
<td>792</td>
<td>53 (51.6-53.4)</td>
<td>48.2 (44-52)</td>
<td>43.2 (40-46)</td>
<td>689 (645-733)</td>
<td>12.8 (10-15.5)</td>
<td>28</td>
<td>127</td>
</tr>
</tbody>
</table>

Data is expressed as arithmetic mean (95% confidence interval of the mean).

ALT was significantly greater in female non-C282Y homozygotes compared to C282Y homozygotes (p = .003)

ALT was significantly greater in male non-C282Y homozygotes compared to C282Y homozygotes (p = .05)

AST was significantly greater in female non-C282Y homozygotes compared to C282Y homozygotes (p = .001)

AST was significantly greater in male non-C282Y homozygotes compared to C282Y homozygotes (p = .007)