C282Y/H63D Compound Heterozygosity Is a Low Penetrance Genotype for Iron Overload-related Disease

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ABSTRACT

Background: Hereditary hemochromatosis (HH) occurs due to mutations in the HFE gene. While the C282Y mutation is the most common genotype reported in HH, other genotypes are found less frequently, indicating variable degrees of penetrance. We studied the penetrance of the C282Y/H63D compound heterozygote genotype in developing clinically significant iron overload.

Methods: We have completed a retrospective analysis on every individual within Newfoundland & Labrador who were diagnosed as C282Y/H63D compound heterozygote between 1996 and 2009 through a molecular genetics study. We collected data for up to 10 years following the initial genotyping using electronic health records, including laboratory values, phlebotomy status, radiologic reports and clinic records. Iron overload status was classified based on the HealthIron study.

Results: Between 1996 and 2009, 247 individuals with available health records tested positive for C282Y/H63D compound heterozygosity. Over the 10 years of our study, 5.3% of patients exhibited iron overload-related disease on the background of documented iron overload. Including these individuals, 10.1% of patients had documented iron overload, 23.1% of patients had a provisional iron overload and the remaining 66.8% of patients had no evidence of iron overload. Only 44 patients had documented phlebotomies, likely based on their severe phenotype at baseline. Despite phlebotomy, the prevalence of iron overload was higher among these patients. The penetrance of compound heterozygosity was also significantly higher among men (P < 0.01).

Conclusion: C282Y/H63D compound heterozygosity is a low penetrance genotype in HH. This is the largest reported cohort of C282Y/H63D compound heterozygotes in North America with an extended follow-up.

Keywords: Cirrhosis; Hemochromatosis; Iron overload; Phlebotomy

INTRODUCTION

Hereditary hemochromatosis (HH) is one of the most common autosomal recessive disorders among individuals of Northern European descent and is characterized by dysregulated iron metabolism (1). In HH, mutations of the HFE gene encoding for an HLA class-I-like protein lead to increased iron absorption, total-body iron overload and secondary tissue damage in a wide range of organs (2,3). There are two main point mutations within the HFE gene: C282Y, which accounts for 80% to 90% of HH cases, and H63D, which is associated with a milder form of the disease representing 4% to 7% of HH cases (4). Non-HFE mutations, such as pathogenic variants of genes encoding for HJV (hemojuvelin), HAMP (hepcidin), and TFR2 (transferrin receptor 2) have also been implicated in HH but are much less common than the HFE variants (5).

Our previous observational study in Newfoundland and Labrador showed that both H63D and C282Y homozygotes had a significantly higher transferrin saturation compared to the wild-type HFE gene, and these genotypes were independent predictors of higher iron saturation levels (6). However, our subsequent studies have identified low penetrance for both the C282Y and H63D homozygous genotypes in developing clinical iron overload and associated end-organ damage (7,8). Even with long-term follow-up, most of these patients do not develop any significant iron overload-related disease (7,8). The absence of clinically overt sequelae even in individuals with pathologic homozygous recessive genotypes suggests variable penetrance of the HFE mutations (7,9).

The penetrance of C282Y/H63D compound heterozygosity has not been studied in any North American cohorts. The prevalence of this mutation is 2% to 5% among individuals of Northern European descent (10–12). Previous cohort studies in Australia suggested low penetrance of the C282Y/H63D compound heterozygous genotype (13,14). Despite having higher serum iron indexes than HFE wildtype controls, these patients did not develop significant end-organ damage (14). However, the incidence of cirrhosis was higher among these patients in the presence of concurrent risk factors like chronic alcohol use or hepatitis C virus (HCV) infection (14,15).

The purpose of our study was to describe the penetrance of the C282Y/H63D compound heterozygote genotype in Newfoundland and Labrador, Canada. We also included an extended follow-up of up to 10 years to further characterize the natural progression of HH in C282Y/H63D compound heterozygotes.
METHODS

This study was approved by the Health Research Ethics Board of Memorial University in St. John’s, Newfoundland. All subjects were from the Province of Newfoundland and Labrador (NL), comprised predominantly of Caucasians from Irish and English descent. This is a retrospective analysis of all individuals within NL who were diagnosed as C282Y/H63D compound heterozygotes between 1996 and 2009 through molecular genetics testing. The indication for genotyping was not stated on the requisition. There is only one laboratory in the province that performs genotyping of the HFE gene, ensuring we included every eligible patient in the province of NL.

Electronic health records were used to collect data on age, sex, ferritin, transferrin saturation, serum iron, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, albumin, international normalized ratio (INR), hemoglobin A1C, fasting triglycerides, liver biopsy results and diagnostic imaging results. To avoid including information from partially treated individuals, data collected within 6 months of the molecular genetics study were considered to be the baseline. For optimizing the risk assessment for developing metabolic diseases and subsequent fatty liver disease, the highest hemoglobin A1C and fasting triglycerides levels throughout the study period were recorded. Follow-up data were collected annually for up to 10 years as they were available within the electronic health records. Data corresponding to therapeutic phlebotomy and any organ damage related to hemochromatosis were also recorded as available. In addition, data on concurrent liver diseases were recorded for all individuals. Since these represented a very small fraction of the total study population, having concurrent liver disease did not exclude patients from the final analysis to represent a real-world scenario. Alcohol use disorder was also noted as a risk factor for concurrent liver disease, and this was described as per established criteria by the National Institute of Alcohol and Alcoholism (NIAA) (16).

Iron overload categories were classified based on previously published definitions from the HealthIron Study (11) (Table 1). Evidence of iron overload-related disease was the most severe category and required both documented iron overload with concurrent development of hepatocellular carcinoma, cirrhosis or fibrosis on liver biopsy, tenderness/effusion of the second and third metacarpophalangeal joints, elevated AST (>45 IU/L) or ALT (>40 IU/L) levels, or by the diagnosis of symptoms associated with HH by a physician. Transient elastography was unavailable to assess for hepatic fibrosis during this study. Documented iron overload was defined by serum ferritin >1000 μg/L or hepatic iron staining 3 or 4 on liver biopsy. Magnetic resonance imaging techniques to quantify hepatic iron content or measuring iron per gram of dry weight in biopsy samples were unavailable in our study centres. Provisional iron overload was defined by elevated serum ferritin and transferrin saturation: >300 μg/L and >55% for men and post-menopausal women, >200 μg/L and >45% for premenopausal women, respectively. Patients who did not meet the criteria for either documented or provisional iron overload were considered to have no evidence of clinically significant iron overload.

The data were summarized using descriptive statistics. Incidence and prevalence of the various iron overload categories were calculated using the total number of subjects in the study as the denominator. All statistical analyses were performed with Prism 8 (GraphPad, California, USA) and all figures were designed with Adobe Photoshop CC (Adobe, California, USA). Comparison of mean was done by one-way analysis of variance and statistical independence was assessed with a contingency table and chi-square analysis. A P < 0.05 was considered to be statistically significant for all quantitative analysis.

RESULTS

Within the province of NL, HFE genotyping identified a total of 275 individuals to be C282Y/H63D compound heterozygotes between 1996 and 2009. Upon reviewing the charts of these patients, 28 individuals were excluded from our study. Among them, 22 patients had no available medical records, 5 patients had a duplicate medical record, and one patient was actually a C282Y heterozygote only. Therefore, the data collection and subsequent analysis was conducted on 247 individuals who were C282Y/H63D compound heterozygotes (Figure 1). Out of these patients 63% were male (n = 155) and 37% were female (n = 92). The mean age of the entire study cohort at the time of genotyping was 50 ± 0.83 years. The mean follow-up duration was 6.8 ± 2.4 years.

The baseline characteristics of all participants are listed in Table 2, separated based on their iron overload categories. There were no differences between the groups in terms of baseline bilirubin, albumin or INR. There were also no differences between the highest documented hemoglobin A1C and fasting triglyceride levels. However, there were significant differences (P < 0.001) in terms of ferritin, transferrin.

Table 1. Iron overload categories adapted from HealthIron Study

| Iron overload categories | Clinical findings or laboratory measures |
|--------------------------|-----------------------------------------|
| No evidence of iron overload | Serum ferritin and transferrin saturation lower than the threshold for Provisional or Documented iron overload |
| Provisional iron overload | 1. Elevated serum ferritin (>300 μg/L for men and post-menopausal women, >200 μg/L for premenopausal women) AND 2. Elevated transferrin saturation (>55% for men, >45% for women) |
| Documented iron overload | 1. Increased iron content shown by hepatic iron staining 3 or 4 OR 2. Serum ferritin >1000 μg/L at baseline with documented venesection |
| Iron overload-related disease | Meet the criteria for documented iron overload plus at least one of the following: 1. Hepatocellular carcinoma 2. Cirrhosis or fibrosis on liver biopsy 3. Tenderness/Effusion of the second and third metacarpophalangeal joints 4. Elevated aspartate aminotransferase (>45 IU/L) or alanine aminotransferase (>40 IU/L) 5. Diagnosis of symptoms associated with hereditary hemochromatosis by a physician |
saturation, serum iron, AST and ALT levels (Table 2). Five patients with documented iron overload had liver biopsies that confirmed increased iron content. This was based on hepatic iron staining of at least a Grade 3 level, which is in keeping with the criteria from the HealthIron study (11) (Table 1). One patient with provisional iron overload and three patients with no evidence of iron overload also had liver biopsies. They all had Grade 1-2 hepatic iron staining and as a result were not considered to have a pathological iron overload on biopsy.

At the time of genotyping, 4% of all patients had features of iron overload-related disease on the background of documented iron overload. These patients had a mean ferritin of 1866.1 ± 426.7 at baseline. Including those with iron overload-related disease, 8.1% of all patients met the criteria for documented iron overload, while an additional 16.2% met the criteria for provisional iron overload. The remaining 75.7% of all patients at baseline did not show any evidence of iron overload (Figure 2A). When the data were analyzed at the end of follow-up, the proportion of patients with iron overload-related disease on the background of documented iron overload increased to 5.3%. Including those with iron overload-related disease, the total number of patients with documented iron overload increased to 10.1%, while the proportion of patients with provisional iron overload increased to 23.1%. The number of patients with no evidence of iron overload at the end of our study period was 66.8% (Figure 2B).

According to the medical records, 44 patients underwent phlebotomy or blood donation after initial genotyping. However, the details on the frequency and duration of phlebotomy were not available. The patients who underwent phlebotomy exhibited a more severe phenotype of iron overload at the time of initial genotyping. These patients had a mean baseline ferritin of 772.9 ± 78.45, while the patients with no documentation of phlebotomy had a mean baseline ferritin of 382.74 ± 40.07. 11.4% of patients who had regular phlebotomy had features of iron overload-related disease at the beginning of our study. Including them, a total of 27.3% of phlebotomized patients met the criteria for documented iron overload, while an additional 25% met the criteria for provisional iron overload. The remaining 47.7% of all patients at baseline did not show any evidence of iron overload (Figure 3A). Despite ongoing phlebotomy or blood donation, these patients continued to exhibit a more severe phenotype of iron overload throughout the following 10 years. At the end of our study period, 13.6% of these patients had features of iron overload-related disease. Including them, a total of 34.1% met the criteria for documented iron overload, while an additional 25% met the criteria for provisional iron overload. The remaining 40.9% of all patients did not have any evidence of iron overload throughout our study (Figure 3B).

Table 2. Baseline characteristics of all patients at the beginning of the study

| Baseline characteristics | Mean ± SEM | Documented iron overload (n = 20) | Provisional iron overload (n = 40) | No evidence of iron overload (n = 187) |
|-------------------------|-----------|----------------------------------|-----------------------------------|---------------------------------------|
| Age at genotyping       | 51.9 ± 3.7| 51.3 ± 1.9                       | 49.9 ± 1.1                        |                                       |
| No. of male patients    | 17        | 28                               | 110                               |                                       |
| No. of female patients  | 3         | 12                               | 77                                |                                       |
| Ferritin (μg/L)         | 1516 ± 255| 558 ± 31.8                       | 295 ± 20.4                        |                                       |
| Transferrin saturation (%) | 54 ± 0.8 | 61 ± 0.4                         | 42 ± 0.2                          |                                       |
| Serum iron (μmol/L)     | 27 ± 2.8  | 32.2 ± 1.4                       | 23.4 ± 0.7                        |                                       |
| Aspartate aminotransferase (IU/L) | 171 ± 70.5 | 36.6 ± 5.4                       | 27.4 ± 1.7                        |                                       |
| Alanine aminotransferase (IU/L) | 136 ± 55.2 | 44.5 ± 4.7                       | 17.5 ± 1.8                        |                                       |
| Bilirubin (μmol/L)      | 25 ± 4.2  | 19.2 ± 3.9                       | 31.7 ± 2.4                        |                                       |
| International Normalized Ratio | 1.1 ± 0.03 | 1.1 ± 0.04                       | 1.1 ± 0.2                         |                                       |
| Albumin (g/L)           | 37.3 ± 1.8| 41.1 ± 0.9                       | 40.9 ± 0.5                        |                                       |
| Hemoglobin A1C (%)      | 7.3 ± 0.3 | 7.1 ± 0.2                        | 7.0 ± 0.3                         |                                       |
| Fasting triglycerides (mmol/L) | 2.1 ± 0.08 | 2.2 ± 0.03                      | 2.1 ± 0.1                         |                                       |

*ANOVA P < 0.001

†Highest recorded value throughout the study period
We also compared the progression of the severity of iron overload throughout the duration of our study between patients with and without evidence of phlebotomy. Without any phlebotomy, two patients developed documented iron overload from no evidence of iron overload at the time of their initial genotyping. Comparatively, among the patients with regular phlebotomy, three patients developed documented iron overload over the course of our study, although they all had provisional iron overload at the time of initial genotyping. Without any evidence of phlebotomy, 15 patients developed provisional iron overload, compared to 2 patients who developed provisional iron overload with regular phlebotomy.

The presence of concurrent liver diseases among all patients throughout the entire study period is listed in Table 3. Among the patients with documented iron overload, one was also diagnosed with autoimmune hepatitis based on their positive anti-smooth muscle antibody serology. However, their liver biopsy showed iron deposition in hepatocytes as well as bile duct epithelia, a specific feature in pathological iron overload from HH (17). The other patient from this group was diagnosed with non-alcoholic fatty liver disease (NAFLD) based on their history of metabolic diseases and hepatic steatosis on ultrasound. While they did not have a liver biopsy, they had persistently elevated ferritin despite documented phlebotomies. There was no history of excessive alcohol use or alcohol use disorder among the patients who had documented iron overload.

Patient age at the time of genotyping was not associated with the risk of developing clinically significant iron overload throughout the study period (\( P = 0.26; \) Table 4). However, the penetrance of compound heterozygosity was significantly higher among men (\( P < 0.01; \) Table 5). There was a 50% increase in provisional iron overload (26.5% versus 17.4%) and a threefold increase in documented iron overload (13.5% versus 4.3%) in men compared to women. To address if there is any change in the iron overload status with menopause, female patients were separated into two groups: older than 50 years of age at the time of genotyping or women who are post-menopausal, and younger than 50 years of age at the time of genotyping or women who are pre-menopausal. Despite the trend toward a greater extent of iron overload in post-menopausal women, this difference was not significant (\( P = 0.13; \) Table 6).
Throughout the follow-up period, four (three male and one female) patients with no evidence of iron overload, five patients (three male and two female) with provisional iron overload and four patients (three male and one female) with documented iron overload were noted to be deceased on their electronic medical records. The cause of death for three of the patients with iron overload-related disease was identified to be hepatocellular carcinoma. One of the patients with provisional iron overload was reported to have died from acute hepatitis from alcohol use and another patient was reported to have died from chronic Hepatitis C infection. The cause of death for the remaining patients was either unavailable or unrelated to iron overload.

DISCUSSION

Our study is the first to describe the penetrance of the C282Y/H63D compound heterozygote genotype within a large North American cohort. With a follow-up duration of up to 10 years...
and a mean follow-up duration of 6.8 ± 2.4 years, we were able to closely monitor the natural progression of this genotype in the development of clinical hemochromatosis.

Our results suggest an overall low clinical penetrance of the C282Y/H63D compound heterozygote genotype. At baseline, only 4% of these patients had features of iron overload-related disease even though they tended to have elevated ferritin levels. This is in keeping with our previous work, which showed higher serum iron indexes in compound heterozygotes compared to C282Y or H63D heterozygotes alone (6). Over the 10 years of follow-up, the proportion of patients with the iron overload-related disease increased to 5.3%. Throughout our study, the most prevalent end-organ damage among patients with the iron overload-related disease was the transient elevation of transaminases as this was present in all 13 patients in this category. This was followed by cirrhosis or fibrosis of the liver (four patients), hepatocellular carcinoma (three patients), and arthropathy of the second and third metacarpophalangeal joints (two patients).

The natural progression of the C282Y/H63D compound heterozygote genotype in our study complements our previously reported findings on C282Y and H63D homozygotes (7,8). Our previous study on C282Y homozygotes with a mean follow-up period of 11.6 years showed documented iron overload in 34.3% of all patients. Among these patients, 18.3% had iron overload-related disease and 5.8% developed cirrhosis (8). In comparison, our study on H63D homozygotes with a mean follow-up period of 4.2 ± 2.6 years showed documented iron overload in 6.7% of all patients. Among these patients, 1.7% had the iron overload-related disease (7). Our collective findings across these studies are in keeping with the prevalence of the different HFE mutations in patients with clinical hemochromatosis (12). C282Y homozygosity is the most commonly found genotype among probands, followed by C282Y/H63D heterozygosity, while H63D homozygosity is the least commonly identified genotype in patients with hemochromatosis (12).

We have reported a real-world experience of the penetrance of this genotype and only a proportion of patients received regular phlebotomies. The decision for phlebotomy was not related to the category of iron overload at baseline, but the patients with documented phlebotomies did have elevated ferritin at the time of genotyping. This was likely due to selection bias as the more severe phenotypes at baseline were likely the ones to get regular phlebotomies throughout our study period. While 11.4% of patients receiving phlebotomy showed iron overload-related disease at baseline, over the course of our study not a single patient progressed from having no evidence of iron overload to developing iron overload-related disease. Among the patients who did not receive regular phlebotomies, two patients developed documented iron overload from no evidence of iron overload at baseline. As such, our data support the current clinical practice guidelines in considering therapeutic phlebotomies in compound heterozygotes (18).

The two previous cohort studies in C282Y/H63D compound heterozygotes showed conflicting results on the development of hemochromatosis in men versus women. Gurrin et al. suggested that in male compound heterozygotes, mean iron indices do not change throughout their study and for

| Table 4. Comparison of the iron overload status throughout the study based on the age of initial molecular genetics study (mean ± SD, ANOVA P = 0.26) |
|---------------------------------------------------------------|
| Iron overload-related disease | Documented iron overload | Provisional iron overload | No evidence of iron overload |
|-------------------------------|--------------------------|---------------------------|------------------------------|
| Age at the time of genotyping (years) | 54.6 ± 19.2 | 52.6 ± 15.1 | 52.2 ± 13.6 | 49.4 ± 12.8 |
| No. of patients | 13 | 25 | 57 | 165 |

| Table 5. Contingency table showing the proportion of patients in the different categories of iron overload separated by gender (Chi-square 9.76, df = 2, P < 0.01) |
|-----------------------------------------------|
| Iron overload-related disease | Documented iron overload | Provisional iron overload | No evidence of iron overload | Total no. of patients |
|-----------------------------------------------|--------------------------|---------------------------|------------------------------|----------------------|
| Male | 6.5% (n = 21) | 13.5% (n = 41) | 26.5% (n = 17) | 60% (n = 93) | 155 |
| Female | 3.3% (n = 4) | 4.3% (n = 16) | 17.4% (n = 72) | 78.3% (n = 72) | 92 |
| All patients | 5.3% (n = 4) | 10.1% (n = 16) | 23.1% (n = 72) | 66.8% (n = 72) | 247 |

| Table 6. Contingency table showing the proportion of female patients in the different categories of iron overload separated by their age greater than or less than 50 years at the time of genotyping (Chi-square 4.14, df = 2, P = 0.13) |
|-----------------------------------------------|
| Iron overload-related disease | Documented iron overload | Provisional iron overload | No evidence of iron overload | Total no. of patients |
|-----------------------------------------------|--------------------------|---------------------------|------------------------------|----------------------|
| Women older than 50 years | 4.4% (n = 3) | 6.5% (n = 11) | 23.9% (n = 32) | 69.6% (n = 32) | 46 |
| Women younger than 50 years | 2.2% (n = 1) | 2.2% (n = 5) | 10.8% (n = 40) | 87% (n = 40) | 46 |
| All patients | 3.3% (n = 4) | 4.3% (n = 7) | 17.4% (n = 9) | 78.3% (n = 9) | 92 |
females, there is an increase in serum ferritin only after meno-
apause (14). In contrast, Walsh et al. reported an increase in
both biochemical expression of iron overload as well as
clinical symptoms in men compared to women (13). Our
findings show a threefold increase in documented iron over-
load among men compared to women. We did not see a sig-
nificant difference according to age or menopausal status as
only one patient under 50 years of age and three patients over
50 years of age had documented iron overload. Although this
subgroup analysis was underpowered, this is in keeping with
the natural progression of hereditary hemochromatosis. Up
to 18% of men and 5% of women have end-organ damage
and disease manifestations appear earlier in men than in
women (19). In women, the clinical symptoms usually ap-
pear in the post-menopausal period, due to a lack of iron
loss during menstruation, pregnancy and lactation offsetting
the increased iron absorption from their HFE gene mutation
(20). In our study, 63% of the entire study cohort were male
patients. The initial genotyping was presumably done based
on some level of clinical suspicion for HH, which was most
likely abnormal serum iron indexes. The known high pene-
trance of HH in men can explain the disparity in our study in
terms of patient selection.

The overall penetrance of the C282Y/H63D genotype
differs between our study and the two previous reports
(13,14). The first study concluded that C282Y/H63D patients
may have high iron indexes, but they do not develop the pro-
gressive disease unless there are associated comorbid factors
like excessive alcohol consumption, metabolic disease, or he-
patic steatosis. However, 28 patients among 91 C282Y/H63D
probands (31%) in this study had clinical symptoms consistent
with hemochromatosis (13). The other cohort study showed
only one out of 82 men and none out of 95 women had the
iron overload-related disease (14). Compared to our study de-
sign, both of these were prospective studies and only the latter
(14) used a similar criteria to categorize the different levels of
iron overload as per the HealthIron Study (11). Both studies
also compared the C282Y/H63D compound heterozygotes to
other HFE genotypes, whereas we looked at the natural pro-
gression of the compound heterozygote genotype alone. The
objective criteria from the HealthIron study that we used to
classify the degree of iron overload may explain the lower
prevalence than was noted by Walsh et al. (13). Gurrin et al.
(14) used a similar criteria to ours and reported higher iron
indices among compound heterozygotes compared to HFE
controls. Their follow-up period of 12 years was also simi-
lar to our study since we followed our cohort for up to 10
years. However, the penetrance of the C282Y/H63 gene in
their study was much lower than our findings likely because
of the differences in the study methodology. Their prospec-
tive study was based on 2.4% of all patients enrolled in the
Melbourne Collaborative Cohort Study and this was a direct
comparison with the HFE wildtype genotype. In comparison,
we retrospectively studied C282Y/H63D compound heter-
zygote patients who were identified by molecular genetic
studies within the province of Newfoundland and Labrador.
This key difference in patient selection is likely the explana-
tion for the variable penetrance of the C282Y/H63 genotype.

Environmental factors and other comorbidities may play a
role in the variable penetrance of the HFE mutations. These
include oral iron intake, excessive alcohol use and other risk
factors for chronic liver disease (13,21). In our study, we
accounted for concurrent liver disease associated with viral
hepatitis, autoimmune etiologies, excessive alcohol intake in
the form of alcohol use disorder and metabolic liver diseases
like non-alcoholic fatty liver. Given the low penetrance of
the C282Y/H63D compound heterozygote gene and the low
cumulative incidence of end-stage liver disease, these factors
were unlikely to play a major role. As a result, we did not ex-
clude patients from our study if they had any concurrent liver
disease to truly reflect a real-world scenario. The two patients
in the most severe category of iron overload were accounted
for with extensive workup to show that their liver disease was
secondary to pathological iron overload.

However, as a retrospective study, we were still limited by
the data available on the electronic medical record. Ferritin
may not be an ideal marker for initial assessment of iron
storage since it is a non-specific acute-phase reactant. In the
absence of liver biopsies, we have used very high serum fer-
ritin (>1000 μg/L) as a marker for documented iron over-
load. Not only is this reflective of current clinical practice,
but other studies in the past including the HealthIron study have
demonstrated this as an effective method of documenting iron
overload (7,8,11,13,14). Given the prevalence of NAFLD, we
needed an objective way to assess our study population for
steatohepatitis. However, transient elastography with a CAP
score was not available at our disposal during the length of
this study. Abdominal ultrasound reports were heterogeneous
and did not always comment of the extent of steatosis. Some
of the other surrogate measurements like weight, body mass
index or waist circumference were also not available for all
the patients. As a result, we measured the highest documented
fasting triglycerides and hemoglobin A1C levels as surrogate
markers for the risk of developing NAFLD. The prevalence
of NAFLD among patients with type 2 diabetes mellitus is 54%
(22). Furthermore, up to 80% of patients with NAFLD also
have dyslipidemia (23).

There is still a possibility that NAFLD was an under-
recognized entity in our study. This could affect our data if
these patients with NAFLD subsequently developed non-
alcoholic steatohepatitis (NASH) in the context of Ferritin
>1000 μg/L. As per the criteria from the HealthIron study,
these patients would have been identified to have iron
overload-related disease (11). However, only 25% of patients
with NAFLD will go on to develop NASH (24,25). Previous
Canadian cohort studies have identified a median Ferritin
level of 145 μg/L (IQR 62-311) among patients with NASH
(26). In our study, we have identified all patients with a
documented diagnosis of NAFLD on their medical records.
Therefore, the proportion of unrecognized NAFLD/NASH
patients who also have Ferritin >1000 μg/L is not likely to
affect the conclusions of our study. If there were unrecognized
NASH patients misclassified as iron overload this would
mean that the penetrance of the C282Y/H63D genotype was
even lower than we reported.

We are the first to study the penetrance of the C282Y/
H63D compound heterozygote genotype within a large
North American cohort. With a follow-up period of up to 10
years, we were able to closely monitor the clinical progres-
sion of hemochromatosis in compound heterozygotes, both
with and without regular phlebotomy. Studies primarily from
Europe and Australia have been used to design the European
(27) and American (18) clinical practice guidelines for
treating patients with hemochromatosis. While the European
guideline suggests investigating other causes of high serum
iron index in C282Y/H63D compound heterozygotes (27),
the more recent guideline from the American College of Gastroenterology published almost a decade later suggests that both C282Y homozygous and compound heterozygous genotypes are at a higher risk of developing clinical hemochromatosis (18). Our findings support this recommendation as we found the penetrance of compound heterozygosity, although low, to be higher than previously reported.

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**Conflict of Interest**
No conflicts to disclose by any of the authors.

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