Presence of Hemochromatosis-Associated Mutations in Hispanic Patients with Iron Overload

Paul Nieves-Santiago, MD; Dilany Cancel, MD; Dialma Canales, MD; Doris H. Toro, MD

Objective: To determine the characteristics of the Puerto Rico Veteran population with iron overload in terms of demographic features, clinical manifestations, and the presence of hereditary hemochromatosis (HH) mutations, and to compare such characteristics in patients with and without HH mutations.

Methods: A retrospective study was conducted in patients with iron overload (transferrin saturation ≥ 45%) who were tested for HH mutations from January 2003 to June 2007. Data collected included age, gender, body mass index, hemoglobin level, platelet count, ferritin level, transferrin saturation, ceruloplasmin, alfa-1 antitrypsin, anti-nuclear antibodies, aspartate aminotransferase, alanine aminotransferase, alfa-fetoprotein, viral hepatitis profile, imaging studies, and comorbid conditions. Patients were grouped according to the results of the commercially available HH DNA mutation analysis as homozygote, heterozygote, compound heterozygote, or negative.

Results: 94 patients were studied. Most patients were male (90/94); the mean age was 60 years. Of the study group, 36% (34/94) was found positive for HH mutations. The most common mutation was H63D, which was found in 85% (29/34) of patients; 4 homozygotes and 25 heterozygotes. C282Y mutation was identified in only 12% (4/34) of patients, of which one was homozygote. A compound heterozygote (C282Y/H63D) was also identified. After analyzing the data for confounding factors, 6 of 29 heterozygotes had no other risk factors for liver disease other than the H63D mutation.

Conclusion: The predominance of H63D mutations in our population deserves further investigation since it considerably differs from other studied populations with iron overload in which C282Y is the most common mutation. [*P R Health Sci J 2011;30:135-138*]

Key words: Hemochromatosis, Iron overload, Genes, Mutations, Hispanics, Puerto Rico

Hemochromatosis is a genetic disorder of iron storage that results in excessive iron deposition in the parenchymal cells of the body. This deposition causes tissue damage and impaired function of organs, especially the liver, pancreas, heart, joints, and pituitary gland (1). It is the most common inherited liver disease in whites and the most common autosomal recessive genetic disorder.

The gene responsible for the disease is named HFE, and is located in chromosome 6. This gene was first discovered in 1996, and consists of a mutation in which tyrosine is substituted for cysteine at the amino acid location 282 (C282). Mutations of the HFE gene are found in nearly 90% of patients with clinical hemochromatosis, most of whom are homozygous (2). Several other mutations have been described; the most common are at locations H63D and S65C.

Several studies have demonstrated that approximately 80 to 100% of patients with the hemochromatosis phenotype are homozygote for the C282Y mutation (3-5). Furthermore, it appears that patients who are heterozygote C282Y/H63D and C282Y/S65C are at increased risk of developing hemochromatosis phenotypically. In a report of asymptomatic patients with iron overload, 57% were homozygous for the C282Y mutation, while 37% were either homozygous for the H63D mutation, heterozygous for the C282Y or H63D mutation, or had compound heterozygosity (6). Several studies suggest that approximately 4 to 7% of patients with the hemochromatosis phenotype have compound heterozygosity (7-9). C282Y/H63D compound heterozygosity has been reported to account for 2 to 5% of cases of phenotypic hemochromatosis with an estimated penetrance of less than 1.5 % (10). Walsh et al demonstrated that patients who were heterozygote did not develop phenotypic hemochromatosis unless a comorbid

Gastroenterology Section, VA Caribbean Heathcare System, San Juan, Puerto Rico

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Address correspondence to: Doris H. Toro, MD, Chief of Medicine, Gastroenterology Program Director, VA Caribbean Heathcare System, Casia # 10, San Juan, Puerto Rico 00921-3201. Email: Doris.Toro@va.gov

feature such as excess alcohol consumption, hepatic steatosis or diabetes mellitus was also present (11).

Hemochromatosis has been associated more recently with mutations at the hemojuvelin (HJV), hepcidin (HAMP) and transferrin receptor 2 (TFR2) genes. Also, it has been associated with mutations of the ferroportin gene (SLC40A1), although most patients with the SLC40A1 mutation demonstrate a distinct iron overload syndrome (12).

Adams et al reported differences in racial prevalence for C282Y homozygosity; whites having the highest prevalence (0.44%) and Asians the lowest (0.000039 %) (13). In the Puerto Rico Hispanic Veteran population, the reported prevalence of hemochromatosis is around 2%, which is similar to that observed in the United States (14).

The objectives of this study were to determine the characteristics of the Puerto Rico Hispanic Veteran population with iron overload in terms of demographic features, clinical manifestations, and hemochromatosis-associated mutations, and compare such characteristics in patients with and without HFE mutations.

Methods

Study population

The study consisted of a retrospective review of the electronic medical records of patients seen at the Hepatology Clinic of the VA Caribbean Healthcare System, San Juan, Puerto Rico. The central laboratory database was reviewed to identify all patients tested for hemochromatosis-associated mutations from January 2003 to June 2007. All patients ≥18 years of age tested for the hemochromatosis-associated mutations as part of the evaluation of suspected liver disease that also had a calculated transferring saturation ≥45% (serum iron/total iron capacity X 100) were included in the study. Patients without hemochromatosis mutation analysis or a transferring saturation <45% within the specified period of time were excluded. The study was approved by the local Institutional Review Board.

Data collection

The electronic medical records of these patients were reviewed for the following information: demographic features, medical history, physical findings, laboratory results, imaging studies, and hepatic biopsy findings when available. Demographic data included age, gender and body mass index (BMI), and documentation of alcohol use. Laboratory data included hemoglobin level, hematocrit, platelet count, ferritin level, transferrin saturation, ceruloplasmin, alfa-1 antitrypsin, anti-nuclear antibodies, aspartate aminotransferase, alanine aminotransferase, alfa-fetoprotein and viral hepatitis profile. Results of the commercially available hereditary hemochromatosis DNA mutation analysis (Quest Diagnostics Nichols Institute, California) were expressed as C282Y/C283Y, C282Y/H63D, H63D/H63D, C282Y/+, H63D/+ and negative accordingly.

Results of imaging studies such as abdominal sonogram, and abdominal computed tomography scan were recorded. All listed comorbid diseases and complications such as ascites, steatosis, hepatocellular carcinoma, diabetes mellitus, sexual dysfunction, arthritis, cardiovascular disease (cardiomyopathy and congestive heart failure) and presence of metabolic syndrome were also recorded.

Statistical analysis

Results are expressed as frequencies, percentages and mean values. Results were analyzed using SPSS Statistical Analysis software. Pair t-test and chi-square tests were used to compare groups with and without genetic mutations. Statistical significance was determined by a $p < 0.05$.

Results

Patient characteristics

A total of 94 patients fulfilled the inclusion criteria. The majority of patients were men (95.7%), with only four women, representing 4.3 % of our study group. Overall, the mean age was 60.2 years (range 40–84). The majority of our patients were between ages of 40-60 years, representing 55.6% of the study population.

Analysis of genetic mutations in the HFE gene

Of the 94 patients with iron overload, 34 (36.2%) were found positive for HFE gene mutations. Figure 1 shows the distribution of these mutations. The most common mutation was H63D, which was found in 85.3% (29/34) of the patients, most of them heterozygotes. The C282Y mutation was identified only in 4 (11.8%) patients. One patient was a compound heterozygote, positive for H63D and C282Y. Only 5 out of the 34 patients were found to be homozygote; four for H63D and one for C282Y.

Clinical and laboratory features

The majority of the population had a hemoglobin value of 15-17g/dl. No statistical difference for hemoglobin levels was identified between HFE positive and negative groups. All patients had a transferrin saturation ≥45 %, with 22.4% of these being greater than 75%. The average ferritin level among all subjects was 1,119 ng/ml (range 60-9141). There were no significant differences for ferritin levels between patients with and without HFE mutations.

Most patients of our study group (80.0%) were overweight or obese. Twenty-eight patients of the 34 (82.4%) patients who were positive for an HFE mutation had a BMI above 25.0 (overweight and obese); however, this was not statistically significant when compared to the group without HFE mutations. No significant differences were observed for type 2

diabetes mellitus, cardiovascular diseases, arthritis and sexual dysfunction in patients with and without HFE mutations.

Hepatitis C virus infection was present in 50.0% (17/34) of patients with HFE mutations. These findings were comparable to the group without identified mutations. Almost half of patients had evidence of prior exposure to hepatitis B, but none had active infection. More than half of the patients at each study group had history of alcohol abuse.

When possible risk factors for liver disease such as viral hepatitis, alcohol abuse and metabolic syndrome were excluded, 7 patients had unexplained iron overload and liver disease. Of these, 6 were heterozygote for H63D and one was homozygote for H63D.

Discussion

We examined the characteristics of a group of Puerto Rico Veteran patients with increased transferrin saturation who tested positive for the HFE mutations. Interestingly, 36% of patients with a transferrin saturation ≥ 45% were found to have HFE genetic mutations.

Hereditary hemochromatosis is most commonly caused by homozygosity for C282Y (2,12). Recent studies estimate that only 1% of patients with hereditary hemochromatosis are homozygotes for H63D (9). In contrast, the most common HFE mutation identified in our population was H63D (1-9). In our study, 5 patients were homozygote for HFE mutations, but only one for the C282Y.

There are no clear data establishing if H63D mutation leads to iron overload and to the phenotypic manifestations of hemochromatosis, but some studies have suggested that the H63D mutation is associated with a milder form of hereditary hemochromatosis (15-17). After reviewing all available data to exclude other etiologies of liver disease and iron overload we identified 7 patients who had no clear etiology for their increased transferrin saturation other than the H63D mutation. Thus, the role of this mutation in the phenotypic manifestation of iron overload warrants further investigation.

Our study had some limitations. It was conducted in a retrospective manner and the study population consisted predominantly of male subjects. Nonetheless, this study reveals interesting data about the presence of genetic mutations in our study population and how they differ from other populations. To our best knowledge this is the first study to characterize HFE mutations in a population with iron overload in Puerto Rico and to describe a Latino population.

This study identified a small group of patients without known etiology for altered liver function tests who were heterozygote for H63D, raising the possibility that individuals who are heterozygotes could develop phenotypic hemochromatosis even in the setting of H63D mutations. Further studies are needed to establish the genetic pool in our general population and the role these mutations may have in the phenotypic manifestations of hemochromatosis.

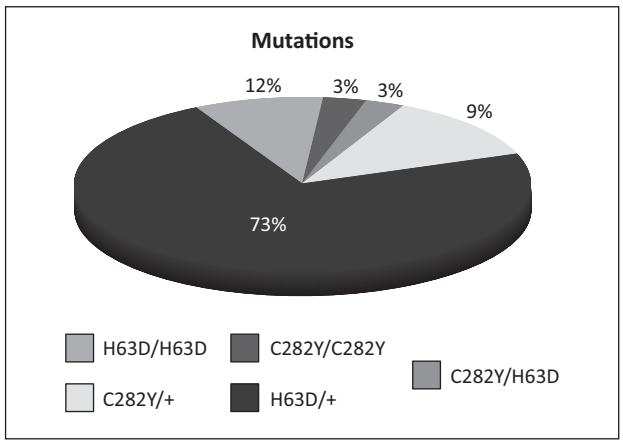

Figure 1. Distribution of HFE mutations among patients with iron overload who tested positive for genetic tests (n=34).

Resumen

Objetivo: Determinar las características de la población de veteranos de Puerto Rico con sobrecarga de hierro en cuanto a datos demográficos, manifestaciones clínicas y la presencia de mutaciones de hemocromatosis hereditaria (HH), y comparar dichas características en pacientes con y sin mutaciones de HH. Métodos: Se realizó un estudio retrospectivo en pacientes con sobrecarga de hierro (saturación de transferrina ≥45%) que se les realizó pruebas genéticas de HH durante el periodo de enero de 2003 a junio de 2007. Los datos recolectados incluyeron la edad, género, índice de masa corporal, hemoglobina, conteo de plaquetas, ferritina, saturación de hierro, ceruloplasmina, antitripsina-1, ANA, enzimas hepáticas, alfa-fetoproteína, pruebas de hepatitis viral, estudios de imágenes, y comorbilidades. Los pacientes se agruparon según los resultados de las prueba genéticas de HH como homocigóticos, heterocigóticos, compuesto, o negativos. Resultados: Se estudiaron 94 pacientes. La mayoría de éstos fueron hombres (90/94); la edad promedio fue de 60 años. En 36% (34/94) de los pacientes se encontró mutaciones de HH. H63D fue la mutación más común, estando presente en 85% (29/34) de los pacientes (4 homocigóticos; 25 heterocigóticos). C282Y se identificó en 12% (4/34) de los pacientes; uno era homocigótico. Se identificó un heterocigoto compuesto (C282Y/H63D). Después de analizar los datos para factores de riesgo, en 6 de 29 pacientes heterocigóticos no se encontró otra posible causa para la enfermedad hepática excepto la mutación H63D. Conclusión: La predominancia de H63D en nuestra población requiere investigación futura, pues difiere marcadamente de lo encontrado en otras poblaciones, donde C282Y es la mutación más común.

References

1. Powell LW, Isselbacher KJ. Hemochromatosis. Harrison's Principles of Internal Medicine. New York: McGraw Hill, 2001: 2257–2261.
2. Harrison H. Adams PC Hemochromatosis, Common genes, uncommon illness? Canadian Family Physician 2002;48:1326-33.
3. Adams PC, Valberg LS, Evolving expression of hereditary hemochromatosis. Semin Liver Dis 1996;16: 47-55.
4. McDonnell S, Preston B, Jewell S, et al. A survey of 2851 patients with haemochromatosis: symptoms and response to treatment. Am J Med 1999;106:619-624.
5. Feder JN, Gnirke A, Thomas W, et al, A Novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13:399-408.
6. Sham RL, Raubertas RF, Braggins C et al. Asymptomatic hemochromatosis subjects: genotypic and phenotypic profiles. Blood 2000;96:3707-11.
7. Beutler E, Gelbrat T, West C, et al Mutation analysis in hereditary hemochromatosis. Blood Cells Mol Dis 1996;22:187-94.
8. Nielsen P, Carpinteiro S, Fischer R, et al. Prevalence of the C282Y and H63D mutations in the HFE gene in patients with hereditary haemochromatosis and in control subjects from Northern Germany. Br J Haematol 1998; 103:842-5.
9. Bacon BR; Diagnosis and management of hemochromatosis, Gastroenterology 1997;113:995-9.
10. Olynyk JK, Cullen DJ, Aguilia S et al. A population-based study of the clinical expression of the hemochromatosis gene, N Engl J Med 1999; 341:718-24.
11. Walsh A, Dixon JL, Ramm GA, et al. The Clinical Relevance of Compound Heterozygosity for the C282Y and H63D Substitutions in Hemochromatosis. Clin Gastroenterol Hepatol 2006;4:1403-1410.
12. Pietrangelo, A, Montosi, G, Totaro, A, et al. Hereditary hemochromatosis in adults without pathogenic mutations in the hemochromatosis gene. N Engl J Med 1999;341:725-732.
13. Adams, PC, Reboussin, DM, Barton, JC, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352:1769-1778.
14. Perez R, Toro DH, Fournier J, Villanueva A. Prevalence of hemochromatosis in the Puerto Rico veteran population. P R Health Sci J. 2007; 26:147-50.
15. U.S. Preventive Services Task Force. Clinical Guidelines .Screening for Hemochromatosis: Recommendation Statement. Ann Intern Med 2006;145:204-208.
16. Mura C; Raguenes O; Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. Blood 1999;93:2502-5.
17. Bacon, BR, Olynyk, JK, Brunt, EM, et al. HFE genotype in patients with hemochromatosis and other liver diseases. Ann Intern Med 1999; 130:953-962.