Blood Cells, Molecules and Diseases 63 (2017) 15–20

Contents lists available at ScienceDirect

Blood Cells, Molecules and Diseases

journal homepage: www.elsevier.com/locate/bcmd

GNPAT p.D519G is independently associated with markedly increased iron stores in *HFE* p.C282Y homozygotes

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ARTICLE INFO

Article history:
Submitted 23 October 2016
Revised 8 November 2016
Accepted 11 November 2016
Available online 12 November 2016

Editor: Mohandas Narla

Keywords:
Hemochromatosis
Iron overload
rs11558492
rs1800562

ABSTRACT

Background: GNPAT p.D519G positivity is significantly increased in *HFE* p.C282Y homozygotes with markedly increased iron stores. We sought to determine associations of p.D519G and iron-related variables with iron stores in p.C282Y homozygotes.
Methods: We defined markedly increased iron stores as serum ferritin >2247 pmol/L (>1000 μg/L) and either hepatic iron >236 μmol/g dry weight or iron >10 g by induction phlebotomy (men and women). We defined normal or mildly elevated iron stores as serum ferritin <674.1 pmol/L (<300 μg/L) or either age ≥40 y with iron ≤2.5 g iron by induction phlebotomy or age ≥50 y with ≤3.0 g iron by induction phlebotomy (men only). We compared participant subgroups using univariate methods. Using multivariable logistic regression, we evaluated associations of markedly increased iron stores with these variables: age; iron supplement use (dichotomous); whole blood units donated; erythrocyte units received as transfusion; daily alcohol consumption, g; and p.D519G positivity (heterozygosity or homozygosity).
Results: The mean age of 56 participants (94.6% men) was 55 ± 10 (SD) y; 41 had markedly increased iron stores. Prevalences of swollen/tender 2nd/3rd metacarpophalangeal joints and elevated aspartate or alanine aminotransferase were significantly greater in participants with markedly increased iron stores. Only participants with markedly increased iron stores had cirrhosis. In multivariable analyses, p.D519G positivity was the only exposure variable significantly associated with markedly increased iron stores (odds ratio 9.9, 95% CI [1.6, 60.3], p = 0.0126).
Conclusions: GNPAT p.D519G is strongly associated with markedly increased iron stores in p.C282Y homozygotes after correction for age, iron-related variables, and alcohol consumption.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; SD, standard deviation; SF, serum ferritin.

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http://dx.doi.org/10.1016/j.bcmd.2016.11.009
1079-9796/

1. Introduction

HFE hemochromatosis is an autosomal recessive condition attributed to homozygosity for the p.C282Y mutation (rs1800562) of the *HFE* gene (chromosome 6p21.3) [1,2]. p.C282Y homozygosity occurs in 0.3–0.6% of persons of European descent and accounts for ~90% of hemochromatosis iron phenotypes in whites [2,3]. Decreased hepcidin expression and consequent excessive iron absorption cause iron overload in p.C282Y homozygotes [4], although clinical penetrance of iron overload in many p.C282Y homozygotes is mild [5,6]. Among persons diagnosed to have hemochromatosis and p.C282Y homozygosity in non-screening venues, the prevalence of iron overload and associated complications such as diabetes, other endocrinopathy, cirrhosis, primary liver cancer, and cardiomyopathy is relatively high [3]. In contrast, the results of population screening studies in whites in European and derivative countries reveal that the proportions of p.C282Y homozygotes who have or develop iron overload and related complications vary but are relatively low [5–8]. Thus, p.C282Y homozygosity is necessary but not sufficient to cause hemochromatosis iron phenotypes [9].

Gender, age, diet, and blood loss account in part for iron phenotype variability in persons with hemochromatosis [10–12]. Modifier mutations in non-*HFE* iron-related genes described to date explain iron phenotype variability in a small proportion of p.C282Y homozygotes [3,13]. Accordingly, it is assumed that other acquired or environmental factors and non-*HFE* polymorphisms of unreported or unconfirmed effect on iron absorption or iron stores account for much of the remaining unexplained iron phenotype variability among p.C282Y homozygotes [14].

GNPAT (chromosome 1q42.2) encodes the peroxisomal enzyme glyceronephosphate O-acyltransferase [15]. The *GNPAT* polymorphism p.D519G (rs11558492) is a candidate modifier of iron phenotypes in p.C282Y homozygotes [16,17]. In an initial p.D519G report, we described 35 men with p.C282Y homozygosity who did not report moderate or heavy daily alcohol consumption [16]. Positivity for p.D519G was significantly greater in the 22 men with markedly increased iron stores than in the 13 other men with normal or mildly elevated iron stores [16]. These results suggested that p.D519G modifies iron phenotypes in men with p.C282Y homozygosity without a history of moderate or heavy daily alcohol consumption either by enhancing iron absorption or because p.D519G is linked to a putative iron absorption promoter on chromosome 1q.

To learn more, we evaluated the significance of *GNPAT* p.D519G as a putative modifier of iron phenotypes in 56 participants with p.C282Y homozygosity who were evaluated with exome sequencing. The present cohort (n = 56) included the original 35 men, of whom 22 had markedly increased iron stores [16], 12 other men (11 of whom had markedly increased iron stores) who were excluded from our initial report because they reported moderate or heavy daily alcohol consumption [16], and 9 additional p.C282Y homozygotes (6 men, 3 women) whose exome sequencing had not been performed at the time of our initial report [16]. Of the 9 additional subjects, 8 had markedly increased iron stores (5 of the 6 men and all 3 women). We applied univariate statistical techniques to compare general observations in the 41 participants with markedly increased iron stores to those of the 15 other participants who had normal or mildly elevated iron stores. Using multivariable statistical techniques, we determined the significance of available variables, including positivity for p.D519G, which could contribute to the development of markedly increased iron stores. We discuss the present results in the context of other reports of p.D519G and iron phenotypes in persons with p.C282Y homozygosity and other *HFE* genotypes.

2. Methods

2.1. Study performance

This study was performed in accordance with the Declaration of Helsinki. Approval was obtained from appropriate review boards at: University of California, Irvine; University of Western Ontario; QIMR Berghofer Medical Research Institute; Rochester General Health System; the Cancer Council Victoria; and Department of Veterans Affairs Long Beach Healthcare System. Written informed consent was obtained from all participants. Consortium study sites identified clinical practice settings associated with the investigators or by population screening through the Hemochromatosis and Iron Overload Screening (HEIRS) Study and a study of the Prevalence of Iron Overload and Frequency of the Hemochromatosis Gene conducted at the Department of Veterans Affairs Long Beach Healthcare System.

2.2. Clinical and laboratory data collection

Information on demographics, blood donation, alcohol consumption, clinical observations, biochemical tests, and interpretation of liver biopsy specimens was obtained from medical records of participants identified through clinical practices and from the National Institutes of Health BioLINCC biorepository for HEIRS Study participants [16]. Observations recorded at diagnosis of hemochromatosis included: age; sex; diabetes diagnoses; use of iron supplements; lifetime units of whole blood donation; lifetime units of erythrocytes received as transfusion; estimated daily alcohol consumption, g; body mass index; swollen/tender 2nd/3rd metacarpophalangeal joints; serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST); and biopsy-proven cirrhosis. Elevated ALT and AST was defined as >0.67 μkat/L (>40 IU/L) for both.

2.3. Exome sequencing

Quality control of sample DNA, exome capture and sequencing, and performance of sequence kernel association tests were performed at the University of Washington (Seattle, WA) as described in detail elsewhere [18].

2.4. Participant selection

Inclusion criteria included: 1) p.C282Y homozygosity confirmed at exome sequencing; 2) participant was unrelated to other participants; 3) participant did not withdraw consent; and 4) participant did not refuse blood storage. Participants with markedly increased iron stores included men and women. By design, all participants with normal or mildly elevated iron stores were men, in order to avoid possible confounding effects of unquantifiable iron losses in women [16]. The participant selection schema is displayed in Fig. 1.

2.5. Classification of iron phenotypes

Induction phlebotomy was defined as the amount of iron removed to achieve serum ferritin (SF) <112.4 pmol/L (<50 μg/L) [19]. Criteria for markedly increased iron stores were: SF >2247 pmol/L (>1000 μg/L) at diagnosis and either hepatic iron >236 μmol/g dry weight or iron >10 g by induction phlebotomy [20]. Criteria for normal or mildly elevated iron stores were: SF <674.1 pmol/L (<300 μg/L) or either age ≥ 40 y with iron ≤2.5 g iron by induction phlebotomy or age ≥ 50 y with ≤3.0 g iron by induction phlebotomy [16].

2.6. Statistics

We used observations for 56 participants in the present analyses. For one participant who reported having received erythrocyte transfusions of unknown number, we imputed a value of one unit of erythrocyte transfusion. Descriptive data are displayed as enumerations, percent (n), mean ± 1 standard deviation (SD), or odds ratios with 95% confidence limits obtained by inverting the Wald Test of the null hypothesis. To compare characteristics of those with markedly increased iron stores to those of participants with normal or mildly elevated iron stores we