Inheritance of two *HFE* mutations in African Americans: Cases with hemochromatosis phenotypes and estimates of hemochromatosis phenotype frequency

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Purpose: Two unrelated African Americans had hemochromatosis phenotypes and genotypes. We sought to identify origins of their *HFE* mutations and estimate frequencies of similar cases. **Methods:** *HFE* and HLA genotyping were performed in index cases and family members. *HFE* genotypes of 1,373 African American controls in five regions were tabulated. **Results:** Index cases had C282Y/C282Y and C282Y/H63D, respectively; each corresponding Ch6p was likely of Caucasian origin. In controls, frequencies of hemochromatosis-associated genotypes were as follows: C282Y/C282Y, 0.00011; C282Y/H63D, 0.00067; and H63D/H63D, 0.00101. **Conclusions:** Penetrance-adjusted estimates indicate that ~9 African Americans per 100,000 have a hemochromatosis phenotype and two common *HFE* mutations. Hemochromatosis-associated genotype frequencies varied 11.7-fold across regions. **Genetics in Medicine, 2001;3(4):294–300.**

Key Words: iron, iron overload, hemochromatosis, African American, *HFE*

Most cases of primary iron overload in African Americans are phenotypically and genotypically distinct from hemochromatosis in whites of northern European descent.^{1–6} However, some African Americans with primary iron overload are heterozygous for C282Y or H63D,^{4–6} the most common mutations of the hemochromatosis-associated *HFE* gene on Ch6p.⁷ Inheritance of two common *HFE* mutations has not been reported in African Americans with primary iron overload, although some African Americans in control populations have been reported to have “typical” or common hemochromatosis-associated *HFE* genotypes C282Y/C282Y, C282Y/H63D, or H63D/H63D.^{8,9} We report the cases of two African American men who had hemochromatosis clinical phenotypes and the *HFE* genotypes C282Y/C282Y and C282Y/H63D, respectively. HLA haplotype analysis in the present cases and a review of HLA haplotype frequencies in various populations made it possible to determine the probable ethnic/racial origins of the *HFE* mutations in both of the present patients. We also tabulated *HFE* mutation analysis data from five African American control populations in various regions of the United States. This permitted estimation of the frequencies with which two common *HFE* mutations would appear in African Americans

who, thus, would be susceptible to developing a hemochromatosis clinical phenotype.

PATIENTS AND METHODS

Case Reports

Patient 1

A 49-year-old man who identified himself as an African American had impotence for 6 months associated with a subnormal serum testosterone concentration of 20.3 $\mu\text{g/L}$ (203 ng/dL); serum concentrations of luteinizing and follicle-stimulating hormones were <0.3 IU/L (<0.3 mIU/mL), respectively. Magnetic resonance imaging of the pituitary did not reveal a significant abnormality. There was no evidence of diabetes mellitus, hypothyroidism, or arthropathy. His erectile dysfunction improved with testosterone enanthate therapy, 200 mg monthly, and he was referred for evaluation of hyperferritinemia. For 1 year, he had ingested daily dietary supplements which contained 10 mg of iron; he rarely consumed alcoholic beverages. He had no history of blood loss due to volunteer donation or other causes. Physical examination revealed mild hepatomegaly. These prephlebotomy serum iron measures were obtained after an overnight fast: serum iron concentration 29 $\mu\text{mol/L}$ (163 $\mu\text{g/dL}$), transferrin saturation 57%, and serum ferritin 726 $\mu\text{g/L}$ (726 ng/mL). Serologic reactions for hepatitis B and hepatitis C were normal or negative. A percutaneous liver biopsy specimen revealed grade 4 intrahepatocytic iron deposits which were most prominent in a periportal location. Ferric iron deposits in Kupffer cells were not detected, and hepatic cirrhosis was not present. Hepatic iron

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concentration was 9,424 $\mu\text{g Fe/g}$ dry weight of liver, and hepatic iron index was 3.4. *HFE* mutation analysis revealed that he is a C282Y homozygote; his HLA type is -A3, -B7, 51. Therapeutic phlebotomy of 1 unit weekly ($\sim 500\text{ mL/unit}$; $\sim 200\text{ mg Fe/unit}$) yielded $\sim 3.0\text{ g}$ of iron.

Patient 2

An apparently healthy 20-year-old man who identified himself as an African American underwent testing which revealed prephlebotomy fasting serum iron concentration $30\text{ }\mu\text{mol/L}$ ($166\text{ }\mu\text{g/dL}$), transferrin saturation 89%, and serum ferritin $141\text{ }\mu\text{g/L}$ (141 ng/mL). Testing was performed because his mother was white and had a "classical" hemochromatosis clinical phenotype associated with C282Y homozygosity. He did not have a history of ethanol use, viral hepatitis, or other hepatic disease, ingestion of supplemental iron, or blood loss due to volunteer donation or other causes. His physical examination, complete blood count, serum concentrations of hepatic enzymes, and thyroid-stimulating hormone level were normal. *HFE* mutation analysis revealed that he is a compound heterozygote for C282Y and H63D; his HLA type is -A3, 23, -B14. He was diagnosed to have hemochromatosis and treated with phlebotomy. After four units of phlebotomy, he developed mild anemia and fatigue, and phlebotomy was discontinued. Repeat postphlebotomy testing after an overnight fast revealed serum iron $22\text{ }\mu\text{mol/L}$ ($121\text{ }\mu\text{g/dL}$), transferrin saturation 63%, and serum ferritin $44\text{ }\mu\text{g/L}$ (44 ng/mL). He received no additional treatment and was well 2 years later when he was referred for further evaluation due to his concerns about the diagnosis and prognosis of hemochromatosis. His relatives provided their respective histories and volunteered to undergo phenotyping using serum iron measures and *HFE* genotyping, as indicated.

Definition of hemochromatosis clinical phenotype and common hemochromatosis-associated *HFE* genotypes

We defined a presumed hemochromatosis or primary iron overload phenotype as persistent elevation of transferrin saturation.^{10,11} We defined "typical" or common hemochromatosis-associated *HFE* genotypes as C282Y/C282Y, C282Y/H63D, and H63D/H63D. For the present analysis, we defined the *HFE* genotypes C282Y/*wt* and H63D/*wt* as not being associated with a hemochromatosis phenotype. There are other mutations of the *HFE* coding region mutations associated with a hemochromatosis phenotype.^{12–15} We did not estimate the frequency of a hemochromatosis phenotype based on their occurrence because (1) the *HFE* missense mutations S65C, I105T, and G93R were not detected in 79 African American controls from central Alabama (Barton JC, unpublished data, 1999); (2) the allele frequency of S65C in 324 black southern California residents who stated that they had a single ethnic background was low (0.0077);⁸ and (3) there are no reports of testing for an *HFE* splice site mutation¹⁴ or for either of two *HFE* stop-codon mutations¹⁵ in African Americans or sub-Saharan Africans.

Laboratory methods

Serum transferrin saturation and serum ferritin concentration were quantified using standard automated methods. Genomic DNA obtained from peripheral blood was used for genotyping. The common *HFE* missense mutations C282Y (exon 4; nt 845G $\rightarrow$ A) and H63D (exon 2; nt 187C $\rightarrow$ G) were detected as previously described^{12–16}; analysis for the hemochromatosis-associated nonclassical transferrin receptor gene mutation (*TFR2* exon 6; nt 750C $\rightarrow$ G; Y250X)¹⁷ was performed using a sequence-specific priming PCR reaction modification of the original PCR-RFLP assay¹⁸ and Y250X control specimens kindly supplied by Dr. Clara Camaschella (Università de Torino, Orbassano-Torino, Italy). HLA-A and -B typing was performed using DNA-based methods.¹²

Frequencies of HLA haplotypes in control populations

To determine the probable racial/ethnic origin of the two Ch6p haplotypes in the present index cases, we tabulated HLA-A and -B haplotype frequency data from various control and hemochromatosis populations, including North American Caucasians, Ashkenazi Jews, African Americans, African blacks, and North American Indians (Acton RT, Harman L, Go RCP, unpublished data, 2000).^{19,20} We also reviewed our data on HLA typing among white hemochromatosis probands, African Americans with primary iron overload, and normal control subjects from central Alabama (Acton RT, Harman L, Go RCP, unpublished data, 2000).^{21,22}

Frequencies of common *HFE* mutations in control African American populations

Allele frequencies of C282Y and H63D in control populations of African Americans were tabulated from all identifiable sources^{6,8,9,11,23,24} after a computerized and manual search of the literature. These data were used to estimate the frequencies of C282Y/C282Y, C282Y/H63D, and H63D/H63D genotypes; we compared these estimates with the observed values. As indicated above, it was assumed that uncommon *HFE* mutations do not occur in African Americans.

We used our previous estimates of the penetrance of "classical" hemochromatosis in *HFE* genotype C282Y/C282Y—positive white Americans of 0.8000 in men and 0.5000 in women.¹¹ The estimated penetrance in white Americans with the *HFE* genotypes C282Y/H63D and H63D/H63D is approximately 1%, respectively.^{7,16} It was assumed that penetrance percentages for the latter two genotypes were equal in men and women. We applied these proportions to data from African Americans to estimate the frequency of primary overload attributable to inheritance of common *HFE* mutations. In all calculations, it was assumed that there are equal numbers of men and women and that the estimates are otherwise applicable to African Americans.

Statistical considerations

Frequencies of *HFE* alleles and genotypes were determined by direct counting. General descriptive data are presented as

percentages. *HFE* mutation frequencies estimated from pooled data were calculated after we obtained the sums of abnormal genotypes and total subjects studied in all identifiable studies. Allele and haplotype frequencies were rounded to four significant figures, except in those cases in which the frequency of a genotype was very low. Comparisons between groups were tested for statistical differences using chi-square analysis. An alpha value of <0.05 was defined as significant.

RESULTS

Family history and HLA analyses

The ancestors of patient 1 included persons of African, Caucasian, and native American heritage. His Ch6p haplotypes were assumed to be HLA-A3, -B7; C282Y and HLA-A3, -B51; C282Y. In the case of patient 2, we confirmed the *HFE* genotypes of the index case and his mother; other data from the family are displayed in Figure 1. The paternal Ch6p haplotype of patient 2 was characterized as HLA-A23, -B14; H63D. This haplotype was also detected in his paternal grandfather who identified his racial/ethnic origin as African, Cherokee, Jewish, and Spanish. The maternal Ch6p haplotype of patient 2 was HLA-A3, -B14; C282Y; his mother identified the countries of origin of her ancestors as France, Germany, Irish, and Norwegian.

Frequencies of HLA haplotypes in control and primary iron overload populations

The HLA-A3, -B7 haplotype, identified in patient 1, is considered by many to be part of the ancestral haplotype contain-

ing the *HFE* mutation C282Y. This haplotype is relatively common among North American and Alabama Caucasians and North American Indians, and is less frequently represented among Ashkenazi Jews, North American blacks, and African blacks (Table 1).^{25–28} The second haplotype in patient 1, HLA-A3, -B51, is much rarer than HLA-A3, -B7 among Caucasians and North American Indians, and even less frequent among North American and African blacks (Table 1). The HLA-A23, -B14 haplotype, identified in patient 2, was only detected among Ashkenazi Jews and Alabama Caucasians in population surveys. However, HLA-A3, -B14, the second haplotype in patient 2, occurs more than three times as frequently in North American or Alabama Caucasians than in North American blacks (Table 1).

Frequencies of common *HFE* mutations in control African American populations

We tabulated data from 1,373 African Americans from five geographic regions of the United States. Overall, the estimated C282Y allele frequency was 0.0106 and the H63D allele frequency was 0.0317 (Table 2). Across the five control population groups, the variation in estimated C282Y and H63D allele frequencies was more than threefold. The differences in the estimated frequency values across geographic regions were 11.7-fold for C282Y/C282Y, 8.6-fold for C282Y/H63D, and 11.3-fold for H63D/H63D (Table 3).

The overall estimated frequencies of *HFE* genotypes among the 1,373 African American control cases were as follows: C282Y/C282Y, 0.00011; C282Y/H63D, 0.00067; and H63D/H63D, 0.00101. The overall observed frequency of the genotype C282Y/C282Y, based on the detection of a single case in South Carolina (0.00036), was 3.3-fold greater than that predicted using observed C282Y allele frequencies (0.00011). The overall observed frequencies of the genotypes C282Y/H63D (detected in 1 California and 1 South Carolina control subjects) and H63D/H63D (detected in 2 California and 1 South Carolina control subjects) were approximately the same as those predicted using corresponding allele frequency data.

The estimated frequencies of hemochromatosis clinical phenotypes attributable to inheritance of two common *HFE* mutations based on the overall estimated *HFE* genotype frequencies (Table 3) were 0.00010814 in men and 0.00007214 in women. This represents approximately 11 per 100,000 men and approximately 7 per 100,000 women. Based on the assumption that there are equal percentages of men and women in the African American population, these values suggest that the frequency of a clinical hemochromatosis phenotype attributable to inheritance of two common *HFE* mutations is ~9 per 100,000 in African Americans.

DISCUSSION

Hemochromatosis usually occurs in persons of western European descent, is attributable to mutations of *HFE* and other iron-related genes,^{7,12,17} and is not typically associated with excess iron ingestion. Increased saturation of transferrin usu-

Fig. 1 Pedigree of African American man with a mild *HFE*-associated clinical hemochromatosis phenotype (patient 2). The index case is indicated by an arrow.

Table 1
Frequencies of HLA haplotypes in various control populations^a

Population group	No. of subjects	HLA-A3, -B7 frequency (no. of haplotypes)	HLA-A3, -B51 frequency (no. of haplotypes)	HLA-A3, -B14 frequency (no. of haplotypes)	HLA-A23, -B14 frequency (no. of haplotypes)
North American Caucasians	274,248	0.0430	0.0013	0.0080	0.0
Ashkenazi Jews	258	0.0191	0.0039	0.0163	0.0036
Alabama Caucasians	1210	0.0318	0.0017	0.0108	0.0012
North American blacks	46,406	0.0150	0.0006	0.0025	0.0
African blacks	286	0.0116	0.0	0.0	0.0
North American Indians	8430	0.0428	0.0024	0.0054	0.0

^aData were obtained from these sources: North American Caucasians, North American blacks, and North American Indians¹⁹; Ashkenazi Jews and African blacks^{20,21}; and Alabama Caucasians (Acton RT, Harman L, Go RCP, unpublished data, 2000).²² The national, racial, or ethnic designations of these control populations are those of the corresponding authors. We also searched for documentation of the frequencies of HLA haplotypes HLA-A3, -B14 and HLA-A23, -B14 among Spanish people, because the present second index case had a distant paternal Spanish relative(s). However, we were unable to identify reports of linkage disequilibrium or haplotype frequencies of these HLA haplotypes among persons living in Barcelona,²⁵ Madrid,²⁶ or Cantabria,²⁷ or among Basques.²⁸

Table 2
Allele frequencies of C282Y and H63D in African American control populations^a

Population	No. of subjects	C282Y allele frequency (no. of mutations detected)	H63D allele frequency (no. of mutations detected)
Alabama ^b	238, 232	0.0042 (2)	0.0151 (7)
California	371	0.0110 (8)	0.0510 (38)
Connecticut	56	0.0089 (1)	0.0179 (2)
Michigan ^c	172, 99	0.0145 (5)	0.0152 (3)
South Carolina	536	0.0121 (13)	0.0299 (32)
All subjects	1373, 1294	0.0106 (29)	0.0317 (82)

^aAlabama subjects included 167 women with non-insulin-dependent diabetes mellitus²³; the remainder were unrelated volunteer subjects from the general population (Barton JC, unpublished data, 1999).¹¹ California subjects were persons attending a health appraisal clinic who indicated that they had a single ethnic background.⁸ Connecticut subjects were presumed normal.²³ Michigan subjects were healthy unrelated persons.⁶ South Carolina specimens were obtained from cord blood from anonymous consecutive births; twins were excluded.⁹

^bA total of 238 subjects were assessed for C282Y, and 232 subjects were tested for H63D.

^cA total of 172 subjects were assessed for C282Y, and 99 subjects were tested for H63D.

ally occurs regardless of presence or severity of iron overload.^{10,29} Excess iron deposition occurs in the liver, pancreas, anterior pituitary, joints, heart, and other organs; iron deposits occur predominantly in parenchymal cells and the spleen and marrow are usually spared. Iron overload can cause hepatic cirrhosis, primary liver cancer, diabetes mellitus and other endocrinopathy, arthropathy, cardiomyopathy, and decreased longevity. African iron overload is typically associated with sub-Saharan ancestry, inheritance of putative susceptibility alleles not on Ch6p which are not presently characterized otherwise,^{30,31} and with ingestion of large quantities of dietary iron which contaminates traditional beer.^{30,31} Hyperferritinemia is always present, and increased saturation of transferrin is com-

mon in untreated patients.^{30–32} Increased iron deposition, predominantly in macrophages, occurs in the liver, spleen, bone marrow, heart, and pancreas. Some persons develop hepatic cirrhosis, primary liver cancer, diabetes mellitus and other endocrinopathy, and cardiomyopathy.³¹ Primary iron overload in African Americans is similar to that in sub-Saharan African natives, although *HFE* mutations in African Americans, especially C282Y, occur infrequently (as indicated herein). Most cases are not associated with ingestion of increased quantities of iron, and increased saturation of transferrin occurs irregularly in untreated patients.^{1,3,31}

The present cases demonstrate that a hemochromatosis clinical phenotype associated with inheritance of two common *HFE* mutations sometimes occurs in African Americans. A persistently elevated serum transferrin saturation value measured using a specimen obtained after an overnight fast is a standard definition of a presumed hemochromatosis clinical phenotype in western Caucasian peoples.^{10,29} However, increased transferrin saturation can also occur in African iron overload and African American iron overload,^{1,3,31} in other iron overload disorders (Witte, 1996), after the ingestion of supplemental iron,¹⁰ and in some liver disorders.¹⁰ In many cases, possible causes of elevated transferrin saturation can be distinguished after consideration of the history and physical examination and review of the complete blood count and serum hepatic enzyme measurements. Based on a series of African American primary iron overload cases gathered from a variety of sources, an elevated transferrin saturation value occurred in 10 of 13 cases in which this measurement was reported.³¹ The frequency of increased transferrin saturation values among African Americans in the general population is approximately 0.09,³³ although the percentage of these persons who have iron overload has not been reported.

In the first case, severe hepatic iron overload and hypogonadotropic hypogonadism were present; these are common complications of iron overload in white men who are C282Y homozygotes.^{5,7} In the second case, the pretreatment serum ferritin concentration and the amount of therapeutic phlebot-

Table 3
Frequency estimate of common hemochromatosis-associated *HFE* genotypes in African Americans^a

Study population	No. of subjects	C282Y/C282Y	C282Y/H63D	H63D/H63D
Alabama ^b	238, 232	0.000018	0.00013	0.00023
California	371	0.00012	0.00112	0.00260
Connecticut	56	0.00008	0.00032	0.00032
Michigan ^c	172, 99	0.00021	0.00044	0.00023
South Carolina	536	0.00015	0.00072	0.00089
Overall estimated frequency	1373, 1294	0.00011	0.00067	0.00101

^aThese estimates were obtained using observed C282Y and H63D allele frequencies compiled from 1373 and 1294 African Americans, respectively,^{6,8,9,11,23,24} and are based on the assumption that *HFE* missense mutations other than C282Y and H63D are very rare in African Americans.

^bA total of 238 subjects were assessed for C282Y, and 232 were tested for H63D.

^cA total of 172 subjects were assessed for C282Y, and 99 were tested for H63D.

omy that induced iron depletion demonstrate that he did not have iron overload.³⁴ This finding is consistent with his age and the mild clinical phenotype often associated with C282Y/H63D compound heterozygosity in white Americans.^{5,7,35} Although it has been postulated that a stop-codon mutation in the nonclassical transferrin receptor could account for African iron overload,¹⁷ this mutation was not detected in the present cases, nor in 274 African American controls and 20 African American unrelated primary iron overload patients.¹⁸ Because persistent elevation of transferrin saturation and increased iron absorption are characteristic of western Caucasian persons with *HFE*-associated hemochromatosis mutations regardless of the presence or severity of iron overload,^{10,29} it is assumed that inheritance of the same genotypes in persons of African American descent would be associated with a hemochromatosis clinical phenotype, consistent with the present cases. At present, however, it cannot be determined whether our present index cases may also have a putative African iron overload mutation(s).³⁰

One haplotype of the first index case, HLA-A3, -B7, is common among Caucasians with and without hemochromatosis.^{19,20,22,36} The HLA-A3, -B51 haplotype is not typically associated with hemochromatosis in European populations,³⁶ and has been observed rarely among Caucasians, North American blacks, and North American Indians (Acton RT, Harman L, Go RCP, unpublished observations, 2000).^{19,20,22,36} This haplotype was not observed in a sample of African blacks.²⁰

The paternal haplotype of our second index case, characterized by HLA-A23, -B14; H63D, is rare, and is more likely to have been inherited from Jewish or other Caucasian ancestors. This is consistent with the family history of the second index case, the greater frequency of the HLA-A23, -B14 haplotype in Jews and other Caucasians (Acton RT, Harman L, Go RCP, unpublished observations, 2000),^{19,20} and the world-wide distribution and frequency of H63D.^{37–39} However, we did not detect this haplotype in two other H63D-positive African Americans with iron overload⁵ or among 13 white hemochromatosis probands from central Alabama who also inherited H63D.¹⁶ Similarly, this HLA haplotype is not typically associ-

ated with hemochromatosis in other persons of northern European descent.³⁶ The maternal haplotype in patient 2, characterized by HLA-A3, -B14, occurs more frequently among persons of northern European descent,^{19,20,22} consistent with the present family history. This haplotype occurs with significantly greater frequency among hemochromatosis patients in Sweden, Brittany, and Utah than in corresponding normal control subjects,^{36,40–42} indicating its association with C282Y which also occurs predominantly in northern European peoples.³⁸ Furthermore, C282Y is rare or undetectable in sub-Saharan African populations.^{39,43}

Before discovery of the *HFE* gene, the frequency of hemochromatosis in African Americans was estimated to be 3–6 per 10,000.¹⁰ This is in good agreement with the present estimate of *HFE*-associated hemochromatosis in African Americans (~9 per 100,000) based on C282Y and H63D allele frequencies, reasonable penetrance estimates, and geographic variation in frequencies of *HFE* mutations in African and Caucasian Americans. Small numbers of additional *HFE*-associated hemochromatosis cases may occur in African Americans in some populations, e.g., those who reside in southern California, due to uncommon *HFE* missense mutations such as S65C.⁸ However, S65C was not detected in 79 control African Americans from central Alabama (Barton JC, unpublished data, 1999), and there are no reports of testing for this allele in African Americans from geographic regions other than California and Alabama. Furthermore, it is possible that some African Americans who are double heterozygotes for a common *HFE* allele and a putative African iron overload mutation may have an increased risk to develop primary iron overload, although this is unproven.

These observations have significant implications for routine clinical diagnosis and population screening for primary iron overload in African Americans. The present index cases had elevated transferrin saturation values, suggesting that they would also have been diagnosed in a screening program using this phenotypic criterion. Approximately 90 per 10,000 African Americans have a mean transferrin saturation of 63.4 ± 5.7%,³³ and many African Americans with primary iron over-

load reported thus far also had elevated values of transferrin saturation.^{5,31} In contrast, the present estimates indicate that only ~9 African Americans per 100,000 are likely to have a hemochromatosis clinical phenotype associated with two common *HFE* mutations, consistent with estimates derived from an African study population.³⁹ There is a significant degree of uncertainty regarding the estimates of penetrance used in our calculations due to the different definitions of a hemochromatosis phenotype used in various studies.^{10,11} Nonetheless, it remains likely that hemochromatosis clinical phenotypes associated with two common *HFE* mutations occur infrequently among African Americans. Furthermore, African Americans with primary iron overload usually have a normal *HFE* genotype.^{4–6,31} Taken together, these observations imply that phenotypic testing is much more likely to detect cases of primary iron overload in African Americans than *HFE* genotyping.¹¹ The present data also emphasize that the value of *HFE* genotyping to screen for or confirm the diagnosis of primary iron overload disorders in African Americans may vary significantly according to geographic area and degree of racial admixture.

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