Countries of Ancestry Reported by Hemochromatosis Probands and Control Subjects in Central Alabama

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Objectives: We sought to evaluate the hypothesis that the relatively high *HFE* C282Y allele frequency in White persons in central Alabama (0.0896) is due to a predominance of persons of Irish and Scots descent, and is not attributable to Native American ancestry common in this geographic area.

Design: Eighty evaluable hemochromatosis probands with C282Y homozygosity and 319 White controls reported countries of ancestry of their grandparents. Frequencies of country of ancestry reports were tabulated. The reports were also converted to scores that reflect proportional countries of ancestry in individuals. Using the scores, we computed aggregate country of ancestry indices as estimates of group ancestry composition. Results were compared to those of European populations with C282Y allele frequencies >0.0800.

Results: The respective frequencies of ''British Isles'' and Scotland reports were significantly greater in hemochromatosis probands than in controls. The respective frequencies of ''Europe Not British Isles,'' Italy, and Poland reports were significantly greater in controls. Aggregate ''British Isles'' and Scotland indices were significantly greater in hemochromatosis probands. The ''Europe Not British Isles'' index was significantly greater in controls. Approximately one-quarter of hemochromatosis probands and controls reported ''Native American'' ancestry; the corresponding country of ancestry index was not significantly different in probands and controls. C282Y frequencies >0.0800 were reported from England, Ireland, Scotland, Wales, Brittany, and Denmark.

Conclusions: The present results indicate that hemochromatosis probands with C282Y homozygosity in central Alabama report significantly different countries of ancestry than control subjects. It is unlikely that Native American ancestry is associated with an enrichment of hemochromatosis among adult probands. British Isles ancestry, not exclusively Irish and Scots ancestries, likely accounts for the relatively high C282Y frequency in White persons in central Alabama. (*Ethn Dis.* 2004;14:73–81)

Key Words: Ancestry, C282Y, Iron, *HFE,* Hemochromatosis

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Introduction

Hemochromatosis is a common autosomal recessive disorder that occurs predominantly in Caucasians of northern European descent. Among White persons with hemochromatosis, 60%–100% are homozygous for a common missense mutation of the *HFE* gene on Ch6p (C282Y; exon 4, nt 845 G→A).[1–4] Some observations indicate that C282Y probably originated in Celts.[5–8] Other data suggest that C282Y first arose in Vikings.[8–12] It is probable that Vikings were primarily responsible for dissemination of C282Y in medieval Europe.[8,9,12–14] Thus, the highest allele frequencies of C282Y occur in present-day British Isles and other areas adjacent to the North Sea,[4–13] consistent with Celtic populations and several centuries of Viking exploration, raids, and trading in this area.[15–18] In "post-Viking" centuries, migrations of western Europeans resulted in further spread of C282Y and the occurrence of C282Y and hemochromatosis in the United States and other countries outside Europe.[3,4,13]

A C282Y allele frequency of 0.0598 was observed in American White persons who participated in a cross-sectional population-based study (Third National Health and Nutrition Examination Survey).[19] Less representative cohorts of the general US population had C282Y allele frequencies of 0.0323 and 0.0732, respectively.[1,20] C282Y allele frequencies of 0.0415–0.0896 have been reported in cohorts of White persons in Alabama, California, Connecticut, Maine, Missouri, New Mexico, Oregon, and South Carolina.[21–29] The highest of these frequency values (0.0896) was obtained from the composite results of 2 population-based studies in central Alabama.[21,22]

It has been hypothesized that the relatively high C282Y allele frequency in central Alabama is due to the settlement of this area by a predominance of persons of Irish and Scots descent.[30] In the present study, we sought to evaluate this hypothesis by using questionnaires to obtain information about the countries of ancestry of the grandparents of White adults in central Alabama with hemochromatosis associated with C282Y homozygosity and of White control subjects from the central Alabama general population. We also hypothesized that native American ancestry of central Alabama White adults does not contribute significantly to the occurrence of hemochromatosis in this geographic area, because hemochromatosis phenotypes or C282Y were rarely detected in native Americans in other studies.[3,31] We evaluated the frequency of country of ancestry reports in hemochromatosis and control participants and devised a country of ancestry index to permit quantification and comparison of group ancestry data. We compared these results with the countries in modern Europe in which the C282Y allele frequency is equal to or greater than that reported in central Alabama. We discuss the pertinence of the present observations to: 1) the ancestry of central Alabama White persons with hemochromatosis associated with C282Y homozygosity and

control subjects; 2) the frequency of C282Y in the Alabama general population; and 3) population testing for hemochromatosis.

PATIENTS AND METHODS

General Criteria for Selection of Study Subjects. The performance of this work was approved by the Institutional Review Boards of Brookwood Medical Center and the University of Alabama at Birmingham. All subjects were adults (≥18 years of age) who resided in central Alabama; each identified himself/herself as White. Persons with hemochromatosis were diagnosed in routine medical care in the interval 1992–2002, but were otherwise unselected. We excluded persons of sub-Saharan African or African-American descent because: 1) *HFE* mutations are uncommon among African Americans in central Alabama; [32,33] and 2) the genotype and phenotype of iron overload in most African Americans is dissimilar to that of hemochromatosis in White persons in our geographic area.[33–36]

Hemochromatosis Probands. Hemochromatosis is often first suspected in routine care delivery because patients develop clinical abnormalities typical of iron overload such as elevated serum concentrations of hepatic enzymes, hyperferritinemia, diabetes mellitus, other endocrinopathy, arthropathy, or chronic fatigue.[37,38] A presumptive diagnosis of hemochromatosis was based on demonstration of a hemochromatosis phenotype defined by persistently elevated transferrin saturation (>60% in men, >50% in women).[21,37,38] Iron overload was defined as otherwise unexplained elevation of serum ferritin concentration (>300 ng/mL in males, >200 ng/mL in females), 3+ or 4+ intrahepatocytic iron visualized using Perls' staining, or hepatic iron index >1.9.[21,37–40] Evaluation for iron overload and its complications were performed as described in detail elsewhere.[21,35,37] Serum transferrin saturation and serum ferritin concentration were quantified using standard automated methods. Genotyping for the common *HFE* mutations C282Y (exon 4; nt 845G→A) and H63D (exon 2; nt 187C→G) was performed using genomic DNA obtained from peripheral blood buffy coat.[21,22] By design, all hemochromatosis probands included in the present study were homozygous for *HFE* C282Y. Of persons diagnosed in routine medical care to have a hemochromatosis phenotype as defined above, approximately 80% are homozygous for *HFE* C282Y.[20] Further, we included only the first persons diagnosed to have hemochromatosis and C282Y homozygosity in respective families; they were designated as probands. Eighty-eight hemochromatosis probands responded to our questionnaire, of whom 8 were inevaluable because they did not know the country of ancestry of any of their grandparents. This left a group of 80 probands whose data were deemed evaluable for the present study.

Control Subjects. Control subjects were recruited in 2 groups. The first group consisted of 260 unselected volunteers who completed the present questionnaire (described below); they were recruited from hospital workers, employees of 2 universities, spouses of patients who attended a hematology/medical oncology outpatient clinic, and members of the general public encountered in 2 retail shopping malls. The second group consisted of 83 spouses of persons with cutaneous melanoma and randomly recruited subjects who were interviewed. In both groups, we excluded persons who were known to be relatives of other study participants. A potential control subject was not included in the study because she reported that she had hemochromatosis. We did not evaluate medical histories, perform physical examinations, or assess serum iron measures or *HFE* genotypes in control subjects. These data were pooled to yield a group of 343 unrelated controls, from whom 24 were eliminated because they did not know the country of ancestry of any of their grandparents (as indicated below). This left a group of 319 control subjects whose data were deemed evaluable for the present study.

Questionnaire and Interview Design. A one-page questionnaire was designed to permit each study participant to indicate the countries of ancestry of his/her paternal and maternal grandparents. This method was, in part, modified from previously reported methods by including only the country of birth of grandparents.[41,42] Choices were presented in identical columns below headings for each of 4 grandparents. Most choices were presented alphabetically: Austria, Belgium, Denmark, England, Finland, France, Germany, Greece, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Russia, Scotland, Sicily, Spain, Sweden, Switzerland, and Wales. Additional choices included "Native American" ("Indian"), "Other" (specific country or countries requested), and "Don't Know." Each participant responded by checking appropriate choices and writing in additional countries, as appropriate. The questionnaire or interview permitted study participants to indicate multiple countries of ancestry for each grandparent. Interview participants identified the countries of ancestry of their grandparents to an interviewer, and their verbal reports were tabulated in a manner similar to that recorded by questionnaire responders. We did not perform separate testing to assess the knowledge of any study participants with respect to the geography or history of countries of ancestry presented on the questionnaire or at interview.

For further analysis, we defined the aggregate category "British Isles" as the composite of reports in England, Ireland, Scotland, and Wales categories, and a related aggregate category "Europe Not British Isles." We defined the category "Native American" as the composite of reports from participants who

indicated such ancestry, regardless of Native American tribe or nation. We defined the aggregate category "North, Central, and South American Countries" as the composite of reports in respective countries in the Americas (other than "Native American" reports), and similarly the corresponding aggregate categories for "Near and Middle East Countries" and "African Countries." Reports based solely on association of family names with specific countries were tabulated as "Don't Know." We did not evaluate relationships of country of ancestry to gender of the grandparents or to the paternal or maternal side of the participant's family.

Frequencies of Countries of Ancestry Reports. We tabulated the numbers of participants who reported specific countries of ancestry or aggregate country categories (as defined above) for one or more grandparents. This method was, in part, modified from previously reported methods by including only the country of birth of grandparents.[41,42] This permitted us to make comparisons of the frequencies of countries of ancestry and aggregate categories reported by hemochromatosis probands and control subjects.

Country of Ancestry Indices. The questionnaire and interview reports from each participant were evaluated to yield individual country of ancestry scores which reflect proportional national ancestry. For this, each participant was assigned 4.00 points divided equally among each of his/her 4 grandparents. If a participant indicated 2 or more countries of ancestry for a grandparent, the 1.00 point for that grandparent was divided equally among the respective countries to formulate the scores for individual participants. We also computed aggregate country of ancestry indices for hemochromatosis probands and control subjects as estimates of group ancestry composition. These indices were expressed as the quotient of total individual scores for respective countries and the total number of hemochromatosis probands or control subjects, as appropriate.

Review of C282Y Allele Frequencies in Europe. A manual and computerized literature search was performed to identify reports of C282Y allele frequencies in White persons in countries in Europe. Because the C282Y allele frequency in central Alabama is approximately 0.0896,[21,22] we tabulated only those reports from countries or regions in which the C282Y frequency was >0.0800. We excluded studies which reported C282Y frequency estimates on cohorts of fewer than 50 subjects.

Statistical Considerations. The dataset consisted of observations on 80 evaluable hemochromatosis probands and 319 control subjects. A computer spreadsheet (Excel 2000, Microsoft Corp., Redmond, Wash) and a statistical program (GB-Stat, v. 8.0 2000, Dynamic Microsystems, Inc., Silver Spring, Md) were used to perform the present analyses. In a preliminary evaluation, we determined that the proportions of men and women, mean ages, country of ancestry reports, and frequencies of country of ancestry reports were not significantly different in the 2 control groups. Therefore, we pooled data from the 2 groups for comparison with those of hemochromatosis probands. Frequencies of men and women, clinical abnormalities, and countries of ancestry were counted. General descriptive data are presented as percentages or mean $\pm$ 1 SD. Comparisons between groups were tested for statistical differences using chi-square analysis, Fisher exact test, or 2-tailed Student *t* test, as appropriate. However, Student *t* test can not be used to compare country of ancestry data groups in which all values are 0 (no variability). Accordingly, we arbitrarily assigned a country of ancestry datum of 0.25 for one person in each proband country group for which there were no actual country of ancestry reports. Student *t* test was then performed using this modified data group; estimated values of *P* from these tests are displayed in parentheses. Bonferroni corrected correlation matrices were used to evaluate multiple comparisons of certain data. A value of $p<0.05$ was defined as significant.

RESULTS

Characteristics of Hemochromatosis Probands. There were reports from 80 evaluable probands (47 men, 33 women). Their mean age at the time of diagnosis was 51 $\pm$ 14 years (range 18–78 years). Their mean serum ferritin concentration before treatment was 1358 $\pm$ 1838 μg/L (range 21–9328 μg/L). Iron overload was present in 48 men and 31 women. At diagnosis, 16% had hepatic cirrhosis demonstrated by biopsy, 16% had arthropathy typical of hemochromatosis, 19% had diabetes mellitus, and none had cardiomyopathy. Fifteen percent of men had hypogonadotrophic hypogonadism.

Characteristics of Control Subjects. There were reports from 319 evaluable control subjects (128 men, 191 women). Their mean age at the time of participation in the present study was 43 $\pm$ 16 years (range 18–82 years). The percentage of women in the control group was significantly greater than that in the hemochromatosis probands ($P=.0036$).

General Analysis of Questionnaire and Interview Reports. No person declined to participate in the study, and there were no incomplete, equivocal, or unintelligible questionnaire or interview reports. Some participants reported that they were unaware of their ancestry due to adoption, family estrangement, or disinterest in genealogy. Eighty of 88 hemochromatosis probands (90.9%) and 319 of 343 control subjects (93.0%) provided reports for at least one of 4 grandparents; these differences were not significant ($P=.5039$). Data from these participants were included from further analysis. Thus there were reports from 80 evaluable hemochro-

matosis probands and 319 evaluable control subjects. Among evaluable hemochromatosis probands, the mean number of countries reported was 2.4 ± 1.3 (median 2; range 1–6 reports). The mean number of countries reported by evaluable control subjects was similar (2.4 ± 1.1, median 2; range 1–7 reports; P=.9431).

Frequencies of Country of Ancestry Reports. These data are displayed in Table 1. Most participants reported European countries of ancestry. Frequencies of Scotland ancestry reported by hemochromatosis probands and control subjects were significantly different (40.0% vs 27.0%, respectively; P=.0223). The frequency of "British Isles" and "Europe Not British Isles" ancestry reports tabulated in hemochromatosis probands were not significantly different than in control subjects. The respective frequencies of Italy and Poland ancestry reported by hemochromatosis probands were significantly lower than those in control subjects. The frequency of Sweden ancestry reports were significantly greater in controls (Table 1). Approximately one-quarter of reports in hemochromatosis probands and control subjects indicated "Native American" ancestry.

Country of Ancestry Indices. These data are displayed in Table 2. The Scotland index in hemochromatosis probands was significantly greater than that in control subjects. The aggregate "British Isles" index was significantly greater in hemochromatosis probands. The aggregate "Europe Not British Isles" index in hemochromatosis probands was significantly less than that in control subjects (Table 2).

We evaluated the association of age in hemochromatosis (at diagnosis and at participation in the present study) and in control groups with aggregate country of ancestry indices in "British Isles," "Europe Not British Isles," "North, Central, and South American Countries," "Native American," and "Don't Know" categories using Bonferroni corrected correlation matrices. In all comparisons, there was no significant correlation of age with percentages of reports in these countries of ancestry categories. Because there were significantly more men in the hemochromatosis probands, we conducted t tests for pooled variances to determine whether men or women in the hemochromatosis or control groups were significantly associated with tabulations of the same country of ancestry categories. The results revealed that the proportions of men and women who reported these aggregate country of ancestry categories were not significantly different.

Table 1. Frequencies of country reports in White Adults in Central Alabama*

Country	Hemochromatosis Probands, % (*N*)	Control Subject, % (*N*)	Values of *P*
England	53.8 (43)	43.9 (140)	.1134
Ireland	51.3 (41)	45.1 (144)	.2840
Scotland	40.0 (32)	27.0 (86)	.0223
Wales	7.5 (6)	4.7 (15)	
British Isles	82.5 (66)	75.6 (241)	.1869
Native American	27.5 (22)	23.8 (76)	.4947
Germany	23.8 (19)	33.5 (107)	.0920
France	11.3 (9)	14.1 (45)	.5042
Norway	3.8 (3)	1.6 (5)†	.2025
Netherlands	8.8 (7)	9.1 (29)	.9242
Lithuania	1.3 (1)	0.3 (1)	.3612
Czechoslovakia	1.3 (1)	0.9 (3)	.5930
Spain	1.3 (1)	2.2 (7)	.5003
Austria	0	2.2 (7)	.2060
Belgium	0	0.3 (1)	.7995
Denmark	1.3 (1)	1.6 (5)	.6541
Finland	0	0.3 (1)	.7995
Greece	0	1.3 (4)	.4070
Hungary	0	0.6 (2)	.6388
Italy	0	5.6 (18)	.0161
Poland	0	5.0 (16)	.0286
Portugal	0	0	—
Romania	0	0.6 (2)	.6388
Russia	0	2.5 (8)	.1640
Sicily	0	1.6 (5)	.3246
Sweden	0	4.4 (14)	.0411
Switzerland	0	1.3 (4)	.4070
Europe not British Isles	51.3 (41)	62.7 (200)	.0612
North, Central, and South American countries†	6.3 (5)	2.2 (7)	.0702
Near and Middle East countries	0	0.9 (3)	.5101
African countries	0	0.3 (1)	.7995

* These data include reports by each evaluable study participant; there were 80 evaluable hemochromatosis probands and 319 evaluable control subjects. The category ''British Isles'' was defined as the reports in England, Ireland, Scotland, and Wales categories. The category ''Europe Not British Isles'' is comprised of the composite data from the corresponding individual countries displayed in the present table. Participants who indicated specific ''Native American'' ancestry most frequently reported Cherokee and Creek descent. The ''North, Central, and South America countries'' category (other than ''Native Americans'') included Canada, United States, Virgin Islands, Colombia, and Brazil. The category ''Near and Middle East countries'' included reports about grandparents from Iran, Lebanon, and Syria. The single observation in ''African countries'' represents the Republic of South Africa. Data from subjects who reported one, 2 or 3 ''Don't know'' countries of ancestry were similar in proband and control groups (data not displayed). Chi-square or Fisher exact tests were used, as appropriate; values of $P<.05$ were defined as significant.

† Four hemochromatosis probands and 4 control subjects in this category reported that they had one or more relatives of ''US'' or ''American'' ancestry.

Table 2. Country of ancestry indices in White adults in Central Alabama*

Country	Hemochromatosis Probands (*N* = 80)	Control Subjects (*N* = 319)	Value of *P*†
England	0.9588	0.7755	.2254
Ireland	0.7900	0.6603	.3212
Scotland	0.5463	0.3038	.0241
Wales	0.0750	0.0674	.8473
British Isles	2.3700	1.8069	.0026
Native American	0.3154	0.2998	.8490
Germany	0.2979	0.4610	.0597
France	0.1688	0.1888	.8028
Norway	0.0750	0.0230	.3434
Netherlands	0.0716	0.1089	.2946
Lithuania	0.0500	0.0031	.3574
Czechoslovakia	0.0125	0.0125	.9979
Spain	0.0125	0.0219	.5421
Austria	0	0.0214	(.0556)
Belgium	0	0.0031	(.9982)
Denmark	0	0.0167	(.1273)
Finland	0	0.0063	(.6546)
Greece	0	0.0408	(.0901)
Hungary	0	0.0094	(.4143)
Italy	0	0.1129	(.0002)
Poland	0	0.0512	(.0007)
Portugal	0	0	—
Romania	0	0.0157	(.3469)
Russia	0	0.0439	(.0120)
Sicily	0	0.0392	(.0701)
Sweden	0	0.0674	(.0027)
Switzerland	0	0.0157	(.1572)
Europe not British Isles	0.6883	1.2628	.0001
North, Central, and South American countries	0.2250	0.0533	.1031
Near and Middle East countries	0	0.0408	(.0901)
African countries	0	0.0063	(.6546)

* These data include all reports by each evaluable study participant. The questionnaire and interview reports from each participant were evaluated to yield individual country of ancestry scores which reflect proportional national ancestry. For this, each participant was assigned 4.00 points divided equally among each of his/her 4 grandparents. If a participant indicated 2 or more countries of ancestry for a grandparent, the 1.00 point for that grandparent was divided equally among the respective countries to formulate the scores of individual participants. We also computed aggregate country of ancestry indices for hemochromatosis probands and control subjects as estimates of group ancestry composition. These indices were expressed as the quotient of total individual scores for respective countries and the total number of hemochromatosis probands or control subjects, as appropriate.

The category ''British Isles'' was defined as the reports in England, Ireland, Scotland, and Wales categories. The category ''Europe not British Isles'' is comprised of the composite data from the corresponding individual countries displayed in the present table. Participants who indicated specific ''Native American'' ancestry most frequently reported Cherokee and Creek descent. The ''North, Central, and South America countries'' category (other than ''Native Americans'') included Canada, United States, Virgin Islands, Colombia, and Brazil. The category ''Near and Middle East countries'' included reports about grandparents from Iran, Lebanon, and Syria. The single observation in ''African countries'' represents the Republic of South Africa.

† Student *t* test cannot be used to compare country of ancestry data groups in which all values were 0 (no variability). Accordingly, we arbitrarily assigned a country of ancestry datum of 0.25 for one person in each proband country group for which there were no actual country of ancestry reports. Student *t* test was then performed using this modified data group; estimated values of *P* from these tests are displayed in parentheses.

We also evaluated the correlations of aggregate country of ancestry indices in hemochromatosis probands in "British Isles," "Europe Not British Isles," "North, Central, and South American Countries," "Native American," and "Don't Know" categories using a correlation matrix. "British Isles" indices had significant negative correlations with indices in "Europe Not British Isles," "North, Central, and South American Countries," "Native American," and "Don't Know" categories ($P<.01$, $<.05$, $<.01$, and $<.01$, respectively). In a similar analysis of data from control subjects, "British Isles" indices had significant negative correlations with aggregate indices in "Europe Not British Isles," "Native American," and "Don't Know" categories ($P<.01$, respectively).

HFE *C282Y Allele Frequencies >0.0800 in Europe.* These data are displayed in Table 3. C282Y frequency data were available from population cohorts in most European nations.[3,4,13] We identified C282Y frequency estimates of ≥0.0800 from England, Ireland, Scotland, Wales, Brittany, and Denmark (Table 2).[3,40–49] The C282Y allele frequency reported from Dublin, Ireland by Ryan et al[47] is significantly greater than that observed in Alabama (0.1422 vs 0.0896, respectively; $P=.0381$, chi-square analysis). However, none of the other C282Y allele frequencies displayed in Table 2 are significantly different from that observed in Alabama, including the C282Y frequency in another report from Dublin by Byrnes et al.[48]

DISCUSSION

The present results indicate that England and Ireland are the countries of ancestry reported most frequently by hemochromatosis probands and control subjects in central Alabama. Other countries of ancestry often reported by the present study participants include Scotland and Germany. These results are consistent with historical accounts of early migrations of persons of English, Irish, Scots, and German descent into central Alabama,[53–56] with the national associations of surnames recorded in Alabama Census returns for 1820 and 1830,[57] and with the present composition of the southern United States.[55] In US Census 2000, country of ancestry information (maximum of 2 countries) was reported on a "long form" provided

Table 3. Allele frequencies of *HFE* C282Y >0.0800 in European White populations

Country (City or Region)	Number of Subjects*	C282Y Frequency	Reference
England (East Anglia)	200	0.0850	40
England (Jersey)	411	0.0827	41
England (Oxford)	330	0.1000	42
Ireland (Belfast)	404	0.0990	43
Ireland (Dublin)	109	0.1422	44
Ireland (Dublin)	411	0.1095	45
Scotland (Northeast)	188	0.0842	46
Wales (not stated)	323	0.0867	3
Wales (South)	10,556	0.0823	47
France (West Brittany)	254	0.0945	48
Denmark (not stated)	219	0.0822	49

* The sources of these subjects included: patients referred to Norfolk and Norwich Hospital for reasons unrelated to known manifestations of hemochromatosis and screened anonymously[40]; volunteer blood donors[41]; Caucasoid renal allograft cadaveric donors[42]; volunteers from bone marrow registry[43]; randomly selected control subjects[44]; newborn screening cards[45]; women attending Aberdeen Maternity Hospital for antenatal care[46]; healthy blood donors[47]; unrelated blood donors from ''Finistère sud''[48]; and Danish newborn screening biobank.[49] Exact source of population specimens was not stated in one report.[3]

to one in 6 census participants, and tabulated as numbers of country-specific reports.[58] Thus the data of US Census 2000 cannot be compared statistically with the results of the present study, but the percentages of European countries of ancestry of White Alabama residents compiled in both studies reveal similar trends. In the present study, the largest subgroup of reports in the "North, Central, and South American Countries" category are those of "US" or "American" ancestry. Other participants did not report or know the country of ancestry of their grandparents. These findings are also consistent with trends in the US Census 1990 and US Census 2000, in which the percentages of White Americans who report "American" ancestry are increasing, and the percentages of those who report various European countries of ancestry are decreasing.[58,59]

The significantly greater aggregate Scotland and "British Isles" indices in central Alabama hemochromatosis probands demonstrated in the present study are consistent with C282Y allele frequencies >0.0800 reported in central Alabama[21,22] and in England, Ireland, Scotland, and Wales.[3,43–50] Some of the present hemochromatosis probands and control subjects also reported France as a country of ancestry. However, it was not possible to associate West Brittany (where the allele frequency of C282Y is high)[51] with any of the present questionnaire or interview reports. Although a C282Y allele frequency of >0.0800 was also reported from Denmark,[49] this nation was not reported as a country of ancestry by any hemochromatosis proband in the present study. Conversely, the aggregate "Europe Not British Isles" and Italy and Poland country of ancestry indices were significantly lower in the present central Alabama hemochromatosis probands than in control subjects. This is consistent with the reported frequencies of these ethnic groups in the southern United States,[55] with the US Census of Alabama,[58] and with analyses of racial and ethnic groups in central Alabama (Jefferson County).[30] These observations are in agreement with the C282Y allele frequencies <0.0800 reported in European countries other than those of the British Isles, and in Italy and Poland.[3,4,13,60–64] Some of the present central Alabama probands who reported "Europe Not British Isles" countries of ancestry also had hemochromatosis associated with C282Y homozygosity. This is consistent with the occurrence of hemochromatosis associated with C282Y homozygosity in Europeans who do not reside in the British Isles.[3,4,13,65]

The present results support our hypothesis that Native American ancestry does not contribute significantly to the occurrence of *HFE* C282Y in central Alabama. "Native American" ancestry, especially Cherokee or Creek heritage, was reported by many of the present study participants, and this is consistent with accounts of early Alabama history[53,66–68] and with US Census data on Alabama since 1820.[57–59] However, the corresponding aggregate "Native American" frequency of country ancestry reports and country of ancestry indices were not significantly different in hemochromatosis probands and control subjects. Thus, it appears unlikely that Native American ancestry enriches the frequency of hemochromatosis in central Alabama White adults, and this is consistent with previous observations that hemochromatosis phenotypes or C282Y are rarely detected in native Americans.[3,31]

The present hemochromatosis patients were probands diagnosed in routine medical care because they had symptoms or signs associated with hemochromatosis and iron overload. Persons discovered to have hemochromatosis in population testing programs are significantly less likely to have signs and symptoms of iron overload.[37,69] Thus it is possible that the method of ascertainment of hemochromatosis could be associated with differences in countries of ancestry reporting, although this is unproven. Because our control subjects were not evaluated using serum iron measures or *HFE* mutation analysis, it is possible that some of them may have had hemochromatosis. Our previous population estimates of frequencies of C282Y homozygosity and hemochromatosis phenotypes in central Alabama Caucasians are 0.0070 in the general population and 0.0020 in men.[21,69] Thus it is unlikely that there are significant biases in the present data and results due to the occurrence of previously

undiagnosed hemochromatosis and *HFE* C282Y homozygosity among control subjects. The percentages of men and women differed significantly in hemochromatosis probands and in control subjects. Additional analyses indicated that these differences do not account for differences in country of ancestry reporting. The predominance of men with hemochromatosis in the present study is typical of most hemochromatosis case series of patients diagnosed in medical care using phenotyping.[21,37,69] The predominance of women in the control group could be attributed to greater percentages of women in the venues we selected for recruitment of control subjects. Because no potential participant declined to complete our questionnaire, it is unlikely that there was other bias favoring selection of women as controls. It is unknown whether there is a significant difference in the knowledge of men and women about their respective countries of ancestry, although such a putative effect is not suggested by the results of the present study. Analyses of the 2 study groups indicate that age is not significantly correlated with country of ancestry indices. However, the mean age of hemochromatosis probands was significantly greater than that of control subjects, and hemochromatosis probands on average reported significantly more countries of ancestry than did control subjects. Thus it is plausible that diagnosis of hemochromatosis or greater age is associated with more interest in personal ancestry, although this is unproven.

Of the hemochromatosis probands and control subjects, 11.8% and 7.3%, respectively, did not know the country of ancestry of any of their 4 grandparents, and many others did not know the ancestry of some of their grandparents. Some participants reported that they were unaware of their ancestry due to adoption, family estrangement, or disinterest in genealogy. Other participants could have been incorrect in their reporting. The percentages of evaluable hemochromatosis probands and control subjects who reported in the "Don't Know" category were similar. Altogether, it is unlikely that exclusion of subjects who did not know the country of ancestry of each of their grandparents would significantly change the outcomes of the present study. The overall trends in the country of ancestry indices and in the frequency of country reporting in "British Isles" and "Europe Not British Isles" categories in the present study were similar. This suggests that uncertainty of participants about the exact degree of country of ancestry of some of their grandparents was probably not a significant contributor to the major conclusions of the present study. In addition, the reporting of certain countries of ancestry by individual hemochromatosis probands does not indicate that *HFE* C282Y was necessarily inherited from grandparent(s) of those specific national ancestries.

Taken together, these present results indicate that aggregate "British Isles" country of ancestry index, not exclusively Irish and Scots ancestries, likely explains the relatively high frequency of *HFE* C282Y in central Alabama. However, trends in the present study suggest that significant differences in England, Ireland, Scotland, or Wales aggregate country of ancestry indices between hemochromatosis probands and control subjects could become apparent if greater numbers of persons were evaluated. Nonetheless, these results generally support the hypothesis we sought to evaluate. The present results also indicate that hemochromatosis phenotyping and *HFE* mutation analysis identify subgroups of White persons in central Alabama in which there is significantly different country of ancestry reporting than in White control subjects. The present results are also consistent with associations of Irish/Scots/English ancestry, HLA-DR immunophenotypes, and estimations of a northern European somatic phenotype (eye and hair color, skin reflectance readings) previously reported in central Alabama White persons.[70,71]

Mathematical modeling of a hypothetical hemochromatosis screening program in central Alabama projects that greater frequencies of hemochromatosis would be detected by targeting population subgroups characterized by British Isles ancestry for evaluation.[30] In a hemochromatosis screening program in Massachusetts, 4 of 5 persons diagnosed to have hemochromatosis reported Celtic ancestry.[72] In a multiracial liver clinic population in England, previously undiagnosed C282Y homozygosity was found to be restricted to persons of northern European heritage, particularly those with Celtic ancestry.[73] Taken together, these reports and the present observations support the concept that targeting White persons in central Alabama (or other geographic areas) who have relatively high "British Isles" country of ancestry indices would be an effective strategy to identify high-risk subgroups for hemochromatosis testing using serum iron measures, *HFE* mutation analysis, or both.

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REFERENCES

1. 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.
2. Burke W, Thomson E, Khoury MJ, et al. Hemochromatosis: gene discovery and its implications for population-based screening. *JAMA.* 1998;280:172–178.
3. Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of *HFE* C282Y and H63D mutations. *Genet Test.* 2000;4:183–198.
4. Porto G, de Sousa M. Variation in hemochromatosis prevalence and genotype in national groups. In: Barton JC, Edwards CQ, eds. *Hemochromatosis. Genetics, Pathophysiology, Diagnosis, and Treatment.* Cambridge: Cambridge University Press; 2000:51–62.
5. Simon M, Le Mignon L, Fauchet R, et al. A study of 609 HLA haplotypes marking for the hemochromatosis gene: (1) mapping of the

gene near the HLA-A locus and characters required to define a homozygous population and (2) hypothesis concerning the underlying cause of hemochromatosis-HLA association. *Am J Hum Genet.* 1987;41:89–105.
6. Malfroy L, Roth MP, Carrington M, et al. Heterogeneity in rates of recombination in the 6-Mb region telomeric to the human major histocompatibility complex. *Genomics.* 1997;43:226–231.
7. Lucotte G. Frequency analysis and allele map in favor of the Celtic origin of the C282Y mutation of hemochromatosis. *Blood Cells Mol Dis.* 2001;27:549–556.
8. Raha-Chowdhury R, Gruen J. Localization, allelic heterogeneity, and origins of the hemochromatosis gene. In: Barton JC, Edwards CQ, eds. *Hemochromatosis. Genetics, Pathophysiology, Diagnosis, and Treatment.* Cambridge: Cambridge University Press; 2000: 75–90.
9. Olsson KS, Ritter B. Idiopatatisk hemokromatose. *Tidfsskr Nor Laegeforen.* 1981;29:101.
10. Olsson KS, Kristoffersson R, Sandberg L. *HLA Markers of the Hemochromatosis Gene on the West Coast of Sweden.* Budapest: European Iron Club Meeting; 1989.
11. Ajioka RS, Jorde LB, Gruen JR, et al. Haplotype analysis of hemochromatosis: evaluation of different linkage-disequilibrium approaches and evolution of disease chromosomes. *Am J Hum Genet.* 1997;60:1439–1447.
12. Jazwinska EC. The ancestral haplotype in hemochromatosis. In: Barton JC, Edwards CQ, eds. *Hemochromatosis. Genetics, Pathophysiology, Diagnosis, and Treatment.* Cambridge: Cambridge University Press; 2000:91–98.
13. Fairbanks VF. Hemochromatosis: population genetics. In: Barton JC, Edwards CQ, eds. *Hemochromatosis. Hemochromatosis. Genetics, Pathophysiology, Diagnosis, and Treatment.* Cambridge: Cambridge University Press; 2000:42–50.
14. Logan FD. *The Vikings in History.* 2nd ed. London: Routledge; 1991.
15. Jones G. The Movement South and Southwest to 954: the British Isles, the Frankish Empire, and the Mediterranean. In: Jones G. *A History of the Vikings.* 2nd ed. Oxford: Oxford University Press; 1968:240.
16. Batey CE, Sheehan J. Viking Expansion and Cultural Blending in Britain and Ireland. In: Fitzhugh WW, Ward EI, eds. *Vikings. The North Atlantic Saga.* Washington, DC: Smithsonian Institution Press; 2000:127–141.
17. Morris CD. The Viking Age in Europe. In: Fitzhugh WW, Ward EI, eds. *Vikings. The North Atlantic Saga.* Washington, DC: Smithsonian Institution Press; 2000:99–102.
18. Rice NS. Laid Waste, Plundered, and Burned: Vikings in Frankia. In: Fitzhugh WW, Ward EI, eds. *Vikings. The North Atlantic Saga.* Washington, DC: Smithsonian Institution Press, 2000:116–126.
19. Steinberg KK, Cogswell ME, Chang JC, et al. Prevalence of C282Y and H63D mutations in the hemochromatosis (*HFE*) gene in the United States. *JAMA.* 2001;285:2216–2222.
20. Beutler E, Gelbart T, West C, et al. Mutation analysis in hereditary hemochromatosis. *Blood Cells Mol Dis.* 1996;22:187–194.
21. Barton JC, Shih WWH, Sawada-Hirai R, et al. Genetic and clinical description of hemochromatosis probands and heterozygotes: evidence that multiple genes linked to the major histocompatibility complex are responsible for hemochromatosis. *Blood Cell Mol Dis.* 1997; 23:135–145.
22. Barton JC, Sawada-Hirai R, Rothenberg BE, Acton RT. Two novel missense *HFE* mutations (I105T and G93R) and confirmation of the S65C mutation in Alabama hemochromatosis probands. *Blood Cells Mol Dis.* 1999; 25:146–154.
23. Beutler E, Felitti V, Gelbart T, Ho N. The effect of *HFE* genotypes on measurements of iron overload in patients attending a health appraisal clinic. *Ann Intern Med.* 2000;133: 329–337.
24. Marshall DS, Linfert DR, Tsongalis GJ. Prevalence of the C282Y and H63D polymorphisms in a multi-ethnic control population. *Int J Mol Med.* 1999;4:389–393.
25. Bradley LA, Johnson DD, Palomaki GE, Haddow JE, Robertson NH, Ferrie RM. Hereditary hemochromatosis mutation frequencies in the general population. *J Med Screen.* 1998;5:34–36.
26. McDonnell SM, Hover A, Gloe D, Ou CY, Cogswell ME, Grummer-Strawn L. Population-based screening for hemochromatosis using phenotypic and DNA testing among employees of health maintenance organizations in Springfield, Missouri. *Am J Med.* 1999; 107:30–37.
27. Garry PJ, Montoya GD, Baumgartner RN, Liang HC, Williams TM, Brodie SG. Impact of HLA-H mutations on iron stores in healthy elderly men and women. *Blood Cells Mol Dis.* 1997;23:277–287.
28. Deloughery TG, Wisner TW, Evans AJ, McWilliams JC, Henner WD, Press RD. Molecular detection and gene frequency in control populations of the common Cys282Tyr mutation implicated in hereditary hemochromatosis. *Blood.* 1996;88:490a.
29. MacClenahan K, Moran R, McDermott S, Longshore JW. Prevalence of hereditary hemochromatosis mutations in the upper savannah region of South Carolina. *Proc Greenwood Genet Center.* 2000;19:45–50.
30. Barton JC, Acton RT. Transferrin saturation phenotype and *HFE* genotype screening for hemochromatosis and primary iron overload: predictions from a model based on national, racial, and ethnic group composition in central Alabama. *Genet Test.* 2000;4:199–206.
31. Borwein ST, Ghent CN, Flanagan PR, Chamberlain MJ, Valberg LS. Genetic expression of hemochromatosis in Canadians. *Clin Invest Med.* 1983;6:171–179.
32. Barton JC, Acton RT, Edward CQ, et al. Iron overload in African Americans: phenotypic and genotypic characterization of probands from central Alabama. In: *A Symposium on Molecular Medicine and Hemochromatosis: At the Crossroads.* Bethesda, Md: National Institute of Diabetes and Digestive and Kidney Diseases; 1998:43.
33. Barton JC, Acton RT. Inheritance of two *HFE* mutations in African Americans: cases with hemochromatosis phenotypes and estimates of hemochromatosis phenotype frequency. *Genet Med.* 2001;3:294–300.
34. Barton JC, Edwards CQ, Bertoli LF, Shroyer TW, Hudson SL. Iron overload in African Americans. *Am J Med.* 1995;99:616–623.
35. Barton JC, Harmon L, Rivers C, Acton RT. Hemochromatosis: association of severity of iron overload with genetic markers. *Blood Cells Mol Dis.* 1996;22:195–204.
36. Barton JC, Shih WWH, Sawada-Hirai R, Bertoli LF, Acton RT, Rothenberg BE. Caucasian hemochromatosis mutation Cys282Tyr in African-American iron overload. *J Invest Med.* 1997;45:62a.
37. Witte DL, Crosby WH, Edwards CQ, Fairbanks VF, Mitros FA. Hereditary hemochromatosis. *Clin Chim Acta.* 1996;245:139–200.
38. Barton JC, Barton NH, Alford TJ. Diagnosis of hemochromatosis in a community hospital. *Am J Med.* 1997;103:498–503.
39. Scheuer PJ, Williams R, Muir AR. Hepatic pathology in relatives of patients with hemochromatosis. *J Pathol Bacteriol.* 1962;84: 53–63.
40. Summers KM, Halliday JW, Powell LW. Identification of homozygous hemochromatosis subjects by measurement of hepatic iron index. *Hepatology.* 1990;12:20–25.
41. Roberts DL. Is Celtic skin different [abstact]? *Br J Dermatol.* 1996;135(suppl 47):34.
42. Long CC, Darke C, Marks R. Celtic ancestry, HLA phenotype, and increased risk of skin cancer. *Br J Dermatol.* 1998;138:627–630.
43. Willis G, Jennings BA, Goodman E, Fellows IW, Wimperis JZ. A high prevalence of HLA-H 845A mutations in hemochromatosis patients and the normal population in eastern England. *Blood Cells Mol Dis.* 1997;23:288–291.
44. Merryweather-Clarke AT, Worwood M, Parkinson L, et al. The effect of *HFE* mutations on serum ferritin and transferrin saturation in the Jersey population. *Br J Haematol.* 1998; 101:369–373.
45. Mullighan CG, Bunce M, Fanning GC, Marshall SE, Welsh KI. A rapid method of haplotyping *HFE* mutations and linkage disequilibrium in a Caucasoid population. *Gut.* 1998;42:566–569.
46. Murphy S, Curran MD, McDougall N, Callender ME, O'Brien CJ, Middleton D. High

incidence of the Cys282Tyr mutation in the *HFE* gene in the Irish population—implications for haemochromatosis. *Tissue Antigens.* 1998;52:484–488.
47. Ryan E, O'Keane C, Crowe J. Hemochromatosis in Ireland and *HFE. Blood Cells Mol Dis.* 1998;24:428–432.
48. Byrnes V, Ryan E, Barrett S, Kenny P, Mayne P, Crowe J. Genetic hemochromatosis, a Celtic disease: is it now time for population screening? *Genet Test.* 2001;5:127–130.
49. Miedzybrodzka Z, Loughlin S, Baty D, et al. Haemochromatosis mutations in North-East Scotland. *Br J Haematol.* 1999;106:385–387.
50. Jackson HA, Carter K, Darke C, et al. *HFE* mutations, iron deficiency, and overload in 10,500 blood donors. *Br J Haematol.* 2001; 114:474–484.
51. Jezequel P, Bargain M, Lellouche F, Geffroy F, Dorval I. Allele frequencies of hereditary hemochromatosis gene mutations in a local population of west Brittany. *Hum Genet.* 1998;102:332–333.
52. Merryweather-Clarke AT, Simonsen H, Shearman JD, Pointon JJ, Nørgaard-Pedersen B, Robson KJH. A retrospective anonymous pilot study in screening newborns for *HFE* mutations in Scandinavian populations. *Hum Mutat.* 1999;13:154–159.
53. Pickett AJ. Chapter XII. The English in Georgia. In: *History of Alabama and Incidentally of Georgia and Mississippi, from the Earliest Period. 1851.* Clerk's Office of the District Court of the United States for the Middle District of Alabama. Republished: Birmingham, Ala: Birmingham Book and Magazine Co; 1962:258–269.
54. Southerland HL Jr, Brown JE. *The Federal Road through Georgia, the Creek Nation, and Alabama, 1806–1836.* Tuscaloosa, Ala: The University of Alabama Press; 1989:1–198.
55. Marcum JP, Williams MW. Geography. Population. In: Wilson CR, Ferris W, eds. *Encyclopedia of Southern Culture.* Chapel Hill, NC: The University of North Carolina Press; 1989:555–556.
56. Miller RM. Ethnic Life. Germans. In: Wilson CR, Ferris W, eds. *Encyclopedia of Southern Culture.* Chapel Hill, NC: The University of North Carolina Press; 1989:429–430.
57. Alabama Census Returns 1820 and An Abstract of Federal Census of Alabama 1830. *Alabama Historical Quarterly, Fall Issue, 1944.* Montgomery, Ala: Alabama State Department of Archives and History; 1944:333–513.
58. US Census Bureau. Available at: http://factfinder.census.gov. Accessed on June 27, 2002.
59. US Census Bureau. Available at: http://www.census.gov/population/www/ancestry.html. Accessed on July 7, 2002.
60. Distante S, Berg JP, Lande K, Haug E, Bell H. High prevalence of the hemochromatosis-associated Cys282Tyr *HFE* gene mutation in a healthy Norwegian population in the city of Oslo, and its phenotypic expression. *Scand J Gastroenterol.* 1999;34:529–534.
61. Campo S, Restuccia T, Villari D, et al. Analysis of haemochromatosis gene mutations in a population from the Mediterranean Basin. *Liver.* 2001;21:233–236.
62. Pozzato G, Zorat F, Nascimben F, et al. Haemochromatosis gene mutations in a clustered Italian population: evidence of high prevalence in people of Celtic ancestry. *Eur J Hum Genet.* 2001;9:445–451.
63. Barosi G, Salvaneschi L, Grasso M, et al. High prevalence of a screening-detected, *HFE*-unrelated, mild idiopathic iron overload in Northern Italy. *Haematologica.* 2002;87: 472–478.
64. Moczulski DK, Grzeszczak W, Gawlik B. Frequency of the hemochromatosis C282Y and H63D mutations in a Polish population of Slavic origin. *Med Sci Monit.* 2001;7:441–443.
65. Barton JC, Acton RT, Prasthofer EF, Rivers CA. Hemochromatosis in a Lithuanian with *HFE* C282Y homozygosity and C282Y allele frequencies in the Baltic Sea region. *Eur J Haematol.* 2001;67:263–264.
66. Pickett AJ. *History of Alabama and Incidentally of Georgia and Mississippi, from the Earliest Period. 1851.* Clerk's Office of the District Court of the United States for the Middle District of Alabama. Republished: Birmingham, Ala: Birmingham Book and Magazine Co; 1962:1–685.
67. Williams WL. Ethnic Life. Cherokees. In: Wilson CR, Ferris W, eds. *Encyclopedia of Southern Culture.* Chapel Hill, NC: The University of North Carolina Press; 1989:423–424.
68. Paredes JA. Ethnic Life. Creeks. In: Wilson CR, Ferris W, Eds. *Encyclopedia of Southern Culture.* Chapel Hill, NC: The University of North Carolina Press; 1989:426.
69. Barton JC, Cheatwood SM, Key TJ, Acton RT. Hemochromatosis detection in a multiphasic health screening program at an Alabama forest products mill. *J Occup Environ Med.* 2002;44:745–751.
70. Barger BO, Acton RT, Soong S-J, Roseman JA, Balch CM, Crist W. The association of the HLA-DR4 with malignant melanoma in a Caucasian population from the southern U.S. *Proc Am Assoc Cancer Res.* 1981;22:71.
71. Acton RT, Barger BO, Go RCP, Roseman J, Soong S-J, Balch C. HLA-DR phenotypes predicts onset of melanoma in a subethnic group of Caucasians. *Proc Am Assoc Cancer Res.* 1981;24:189.
72. Smith BN, Kantrowitz W, Grace ND, et al. Prevalence of hereditary hemochromatosis in a Massachusetts corporation: is Celtic origin a risk factor? *Hepatology.* 1997;25:1439–1446.
73. Moodie SJ, Ang L, Stenner JM, et al. Testing for haemochromatosis in a liver clinic population: relationship between ethnic origin, *HFE* gene mutations, liver histology, and serum iron markers. *Eur J Gastroenterol Hepatol.* 2002;14:223–229.