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SLC40A1 Q248H allele frequencies and Q248H-associated risk of non-*HFE* iron overload in persons of sub-Saharan African descent

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Abstract

The ferroportin polymorphism *SLC40A1* Q248H (exon 6, cDNA 744G→T; Gln248His) occurs in persons of sub-Saharan African descent with and without iron overload, and is associated with elevated serum ferritin concentrations (SF). However, the risk of iron overload associated with Q248H has not been defined. We tabulated previously reported Q248H allele frequency estimates in African Americans and Native Africans, and computed the risk of iron overload associated with Q248H in subjects who lacked *HFE* C282Y. The aggregate Q248H allele frequency in 1,038 African Americans in two cohorts from Alabama and one cohort each from Washington, D.C. and California was 0.0525 (95% CI: 0.0451, 0.0652); there was no significant difference in frequencies across these cohorts. The aggregate frequency in 259 Natives from southeast Africa in two cohorts was 0.0946 (95% CI: 0.0694, 0.1198); the difference between the frequencies of these cohorts was not significant. The aggregate Q248H frequencies in African Americans and Native Africans differed significantly (0.0525 vs. 0.0946, respectively; p = 0.0021). There were reports of 24 unrelated African Americans and 15 unrelated Native Africans without *HFE* C282Y who had iron overload. In African Americans, the odds ratio (OR) of Q248H-associated risk of iron overload using 610 C282Y-negative control subjects unselected for SF was 1.57 (95% CI: 0.52, 4.72; p = 0.29). In Native Africans, the OR using 208 control subjects unselected for SF was 1.05 (95% CI: 0.28, 3.90; p = 0.58). We conclude that the frequency of *SLC40A1* Q248H is significantly lower in African Americans than in Native Africans. Although OR estimates of iron overload in African Americans and Native Africans with Q248H were greater than unity, the increased OR were not statistically significant.

Key Words for Indexing

ferroportin; genetics; hemojuvelin A310G; mutation

Introduction

Ferroportin, an iron exporter encoded by *SLC40A1*, occurs on the surfaces of absorptive enterocytes, macrophages, hepatocytes, and placental syncytiotrophoblasts; ferroportin expression is subject to post-translation regulation by hepcidin [1–3]. *SLC40A1* Q248H (exon

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6, cDNA 744G→T (Gln248His)) occurs as a polymorphism in persons of sub-Saharan African descent [4–6], but was not detected in western Caucasians [6]. Several reports suggest that Q248H may affect iron metabolism in humans, although phenotype attributes of persons with Q248H are incompletely defined.

Beutler *et al.* evaluated 278 African American men and 293 women without *HFE* C282Y who participated in a southern California screening program; the mean serum ferritin concentration (SF) of 26 Q248H heterozygotes did not differ significantly from that of wild-type homozygotes. When the distribution of genotypes of these subjects was stratified to SF of more or less than 500 μg/L in men, and more or less than 350 μg/L in women, a Chi-square test for trend was highly significant [5]. Gordeuk *et al.* described twenty-two first-degree family members of Native Africans with iron overload, ten of whom were Q248H heterozygotes; Q248H was associated with significantly higher SF to aspartate aminotransferase ratios and significantly lower hemoglobin concentrations [6]. McNamara *et al.* reported that Q248H was associated with increased SF and lower values of mean corpuscular volume (MCV) in members of African iron overload families [7]. Rivers *et al.* evaluated selected African American participants in the HEIRS Study screening program; the prevalence of Q248H was greater in men with elevated SF levels than in those with normal SF. However, a corresponding difference in Q248H prevalence in women was not observed [4].

Primary iron overload associated with some ferroportin mutations such as *SLC40A1* A77D [8], V162del [9–11], and G490D [12] is characterized by elevated SF, variable percentages of transferrin saturation, and a predominance of iron loading in macrophages [8,9,13]. These phenotype attributes are also characteristic of many African Americans and sub-Saharan Native Africans who have non-human leukocyte antigen-linked or non-*HFE* primary iron overload [4,14–17], some of whom have Q248H [4–6]. This suggests that Q248H could account for primary iron overload in some persons of sub-Saharan African descent. However, the risk of non-*HFE* iron overload in African Americans and Native Africans with Q248H has not been reported [6,7,18].

We tabulated and compared published allele frequency estimates of *SLC40A1* Q248H in African Americans and in sub-Saharan Native Africans [4–7,19,20]. Using these data, we computed the Q248H-associated risk of iron overload in African Americans and Native Americans who lacked *HFE* C282Y. We report the relationship of iron overload to Q248H in persons of sub-Saharan African descent based on the present computations, and the characteristics of an iron-related phenotype associated with Q248H. We discuss these observations in the context of the conservation of ferroportin Q248 or its homologue in multiple species, the projected location of Q248 in human cell membrane ferroportin, and the function of ferroportin Q248H *in vitro.*

Methods

Definition of sub-Saharan ancestry

Persons of sub-Saharan ancestry were defined as either persons living in the U.S. who reported that they were African, African-American, or black, or Native peoples who resided in sub-Saharan Africa [4–6,21]. Herein, we use the term “African Americans” to designate the former, “Native Africans” designate the latter, or the collective term “persons of sub-Saharan African descent.”

Definitions of iron overload and control subjects

We selected previously reported data on probands or index cases with iron overload defined as the demonstration of elevated hepatic iron concentration, excess iron removed by

phlebotomy to achieve iron depletion, or elevated SF without explanation other than iron overload in subjects who had consumed more than 1000 L of traditional beer, as described in detail elsewhere [4–6]. Persons who had iron overload attributable to multiple erythrocyte transfusions were excluded. Control subjects were defined as persons in convenience or population samples who were presumably unrelated to subjects with iron overload; some control subjects were selected or categorized according to SF.

Previous reports of SLC40A1 Q248H genotyping

We performed a computerized and manual search of medical literature to identify reports of Q248H genotyping in persons of sub-Saharan African descent, and compiled these data according to the geographic region of residence of the subjects and presence or absence of *HFE* C282Y.

SLC40A1 and HFE mutation analyses

SLC40A1 Q248H was detected using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), PCR-allele specific oligomer hybridization (ASOH), denaturing high-performance liquid chromatography, or sequence-based typing technique, as described elsewhere in detail [4–6,20,21]. Lack of Q248H was defined as wild-type *SLC40A1*. *HFE* C282Y was detected using PCR-RFLP or PCR-ASOH technique in African Americans, as described in detail elsewhere [4,5,22,23]. Native African subjects were not tested for *HFE* C282Y directly, but were assumed to be negative for *HFE* C282Y based on previous reports that this allele is absent in sub-Saharan Native Africans [24–27].

Further genetic evaluation of African American iron overload index patients with SLC40A1 Q248H

The coding region and immediate 5′ promoter region of *SLC40A1* was sequenced in 24 African Americans with iron overload (Table 2) as previously described [4–6]. Three subjects were found to be heterozygous and one was homozygous for Q248H (Table 2). DNA was available for further analysis in three of these four subjects (one from California, two from Alabama); exons 2, 3, and 4 of the hemojuvelin gene (*HFE2*, *HJV*) were sequenced in these three subjects [28], but analysis of other possible “modifying” genes in these subjects was beyond the scope of the present study.

Homologues of human ferroportin glutamine residue at amino acid 248

We reviewed previously published sequences of amino acids encoded by *SLC40A1* in humans and ferroportin gene homologues in mice, rats, zebrafish, nematodes (*Caenorhabditis elegans*), and thale cress (*Arabidopsis thaliana*) [29,30]. We also obtained amino acid sequences encoded by *SLC40A1* in humans and the sequences of ferroportin homologues in cattle (*Bos taurus*), and western clawed frogs (*Xenopus tropicalis*) using UniGene [31], and compared them using ExPASy SIM [32].

Statistical analyses

Descriptive data are displayed as enumerations, percentages, or allele frequencies (95% confidence intervals (CI)). Q248H allele frequencies were compared using Student’s t test; Bonferroni adjustment was used, as appropriate. Contingency tables were used to evaluate the association of iron overload and Q248H using counts of subjects who were positive (either heterozygous or homozygous) and negative for Q248H. These results were reported as odds ratio (OR), 95% confidence intervals, and Chi-square or Fisher’s exact p-values, as appropriate [33]. Values of $p < 0.05$ were defined as significant.

Results

Population allele frequency estimates for SLC40A1 Q248H

The aggregate allele frequency of Q248H in 1,238 African Americans in six cohorts was 0.0525 (95% CI: 0.0437, 0.0613) (Table 1). The Q248H allele frequencies did not vary significantly across these six cohorts (Student's t test with Bonferroni adjustment). The lowest and highest estimates of Q248H allele frequency were observed in two respective anonymous cohorts of 100 African Americans (Table 1).

We performed a separate analysis of the data from the geographically defined cohorts in Birmingham, AL (two cohorts), and one cohort each from Washington, D.C. and San Diego, CA (Table 1). This yielded a similar aggregate Q248H allele frequency in 1,038 African Americans of 0.0525 (95% CI: 0.0451, 0.0652). There was no significant difference in Q248H allele frequencies across these four cohorts (Student's t test with Bonferroni adjustment).

The aggregate Q248H allele frequency in 259 Native Africans in two cohorts was 0.0946 (95% CI: 0.0694, 0.1198) (Table 2). The Q248H allele frequency was not significantly different in 208 subjects from Zimbabwe (0.1010) and in aggregate data from 51 South Africa and Swaziland subjects (0.0686) ($p = 0.27$; two-tailed Student's t test) (Table 1).

The difference between the aggregate allele frequencies of African Americans and Native Africans was significant (0.0525 vs. 0.0946, respectively; $p = 0.0021$; two-tailed Student's t test).

Odds ratios of iron overload risks associated with SLC40A1 Q248H

In African Americans, we used aggregate data from subjects with iron overload who lacked *HFE* C282Y (n = 24) and African American control subjects without C282Y (n = 1,038) (Table 2). This yielded an OR of 1.76 (95% CI: 0.59, 5.24; $p = 0.23$). We repeated the analysis using only data from 610 C282Y-negative control subjects unselected for SF (n = 610) (Table 2). This yielded an OR of 1.57 (95% CI: 0.52, 4.7; $p = 0.29$) (Table 2). The proportions of male and female control subjects who had Q248H were not significantly different ($p = 0.77$, Chi-square analysis). Likewise, the proportions of men and women with iron overload who had Q248H were not significantly different ($p = 0.051$, Fisher's exact test).

In Native Africans, we used aggregate data from subjects with iron overload (n = 15) and aggregate data from control subjects (n = 259) (Table 2). This yielded an OR of 1.13 (95% CI: 0.31, 4.15; $p = 0.54$). We repeated the analysis using only data from 208 control subjects unselected for SF (n = 208) (Table 2). This yielded an OR of 1.05 (95% CI: 0.28, 3.90; $p = 0.58$). In Native Africans, the proportions of men and women in control and iron overload groups with and without Q248H were not reported [6,21].

In all subjects of sub-Saharan African descent, we used aggregate data from all subjects with iron overload (n = 39) and aggregate data from all control subjects who lacked or were assumed to lack *HFE* C282Y (n = 1,297). This yielded an OR of 1.64 (95% CI: 0.71, 3.77; $p = 0.18$).

Further genetic evaluation of African American iron overload index patients with SLC40A1 Q248H 2

Three of four subjects with iron overload who had *SLC40A1* Q248H (Table 2) were available for additional testing. One of the three, a woman with Q248H heterozygosity, was also heterozygous for hemojuvelin A310G (*HFE2*, exon 4, cDNA 929C→G (Ala310Gly)) and the synonymous *HFE2* mutation 792G→C (Ser264Ser). *HFE2* A310G has been previously described in African Americans with iron overload (allele frequency 0.0196) and in African

American control subjects (allele frequency 0.0720) [28]. This woman reported no family history suggestive of iron overload, but direct study of her family members was not possible. Thus, we cannot determine whether the single *HFE2* A310G allele affects the severity of iron overload in the presence of heterozygosity for *SLC40A1* Q248H.

Homologues of human ferroportin glutamine 248 residue

The glutamine (Q) residue at amino acid 248 in human ferroportin is conserved at the same numeric position in ferroportins of mice, rats, and western clawed frogs. The corresponding amino acid in cattle is arginine (also residue 248). In zebrafish, the corresponding amino acid is histidine (residue 245). In *Arabidopsis*, however, there is a glutamine residue that is homologous to human ferroportin Q248 [29,30]. In *C. elegans*, there appears to be no homology with ferroportin Q248 in humans or the corresponding protein in other species for which sequences were available [29].

Discussion

Estimates of *SLC40A1* Q248H aggregate allele frequency in African Americans in Birmingham, AL, Washington, D.C., and San Diego, CA did not differ significantly. The lowest and highest values of Q248H allele frequency in African Americans were reported in two respective anonymous cohorts. This suggests that there is geographic variability of Q248H allele frequency in African Americans, although there are no reports of Q248H allele frequency estimates for African Americans who reside in regions other than central Alabama, the area of Washington, D.C., or southern California. There is significant geographic variability of the frequencies of other genetic markers pertinent to iron absorption and overload in African Americans [23,34,35].

The *SLC40A1* Q248H aggregate allele frequency in African American cohorts was significantly lower than the aggregate allele frequency Native African cohorts. Admixture with whites is the probable explanation for the lower Q248H frequencies in African Americans, and is consistent with the absence of Q248H in a population sample of 300 Caucasians and a clinical cohort of 25 Caucasians with non-*HFE* iron overload [6]. However, the degree of Caucasian admixture in several African American cohorts described herein [5,6,20] may be lower than that in random population samples of African Americans, because many of the former did not have *HFE* C282Y, a marker for Caucasian admixture in African Americans [18]. Most African Americans are descendants of Bantu-speaking Natives who lived in west Africa [36,37], whereas the present African Natives resided in southeast Africa. Genetic characteristics of Native Africans vary significantly throughout Africa [38,39], although the allele frequency of Q248H in Natives of west Africa has not been reported.

The present results indicate that the risk of iron overload phenotypes in persons with *SLC40A1* Q248H is not significantly increased. Likewise, the association of Q248H with iron overload in Native African families in a logistic regression analysis was not significant [7]. In persons with iron overload, phenotypes defined by hemoglobin concentrations, MCV, and indirect measures of iron status did not differ significantly between index subjects heterozygous for Q248H and those with wild-type *SLC40A1* [6]. Regardless, persons of sub-Saharan African descent with Q248H may be more likely to be evaluated for iron overload than those without Q248H, because some of them with Q248H have higher SF than persons without Q248H [5, 7,20,21]. Population and family studies suggest that primary iron overload in some persons of sub-Saharan African descent may occur as a heritable trait [7,14–16]. Nonetheless, evaluations of *SLC40A1* (including the present results) and other iron-related genes and of inherited erythroid disorders have not demonstrated a predominant abnormality that accounts for non-*HFE* iron overload in African Americans or Native Africans [4–7,19,26,28,40].

Limitations of the present study include the few regions from which African Americans with or without iron overload were available. The numbers of reported African Americans and Native Africans with primary iron overload and *SLC40A1* genotyping are relatively small; a larger sample of subjects with iron overload could provide greater power to detect a significant risk of iron overload in persons with Q248H in future analyses. Our inclusion of African Americans [20] and Native Africans [6] with SF within the respective reference ranges for sex and laboratory as control subjects could have decreased our inability to detect significant OR, although excluding these subjects did not change our OR estimates greatly. Contrariwise, data from some African American control subjects from Alabama and Washington, D.C. we used had been selected previously because they had elevated SF [20]. Excluding these subjects changed OR estimates very little. One woman with iron overload and Q248H heterozygosity was found to be heterozygous for *HFE2* A310G, but iron overload phenotypes are typically caused by homozygosity or compound heterozygosity for *HFE2* alleles [40,41]. Further, *HFE2* A310G occurs with similar frequencies in African Americans with and without iron overload [28].

Distinctive phenotypes of persons of sub-Saharan African descent with *SLC40A1* Q248H are mild, if any. SF is more likely to be elevated in persons with Q248H (mostly heterozygotes) than in persons with wild-type *SLC40A1* [4–7] In one study of African Americans, this SF phenotype attribute of Q248H was significantly more prevalent in men than in women [20]. Some Q248H homozygotes have been identified [5,7,20], and in one study they had significantly higher mean SF than subjects with wild-type ferroportin [5]. These observations could partly explain previous reports that African Americans, especially men, have higher mean SF than do Caucasians, and that African American men have higher mean SF than African American women [5,42]. Hemoglobin, MCV, transferrin saturation, and SF of first-degree family members of iron overload index subjects did not differ significantly between those who were heterozygotes for Q248H and those with wild-type *SLC40A1* [6]. In contrast, McNamara *et al*. reported that Q248H was associated with lower values of MCV in members of African iron overload families [7], an attribute of erythrocytes of zebrafish with ferroportin mutations [30]. In a study of Native African children, there was no persuasive evidence of a protective effect of Q248H against iron deficiency [21].

A glutamine residue at ferroportin 248 or its homologue is conserved across some but not all species. Ferroportin Q248 is projected to lie in the extracellular portion of the molecule between the third and fourth transmembrane helices, but is relatively remote from a cluster of pathogenic residues (A77D, N144H, V162del) that probably occur in or near a binding site [9]. In *Xenopus* oocytes and human kidney epithelial 293T cells, the expression of ferroportin Q248H at the plasma membrane was similar to that of wild-type ferroportin [43,44], and hepcidin binding of ferroportin Q248H did not differ significantly from that of wild-type ferroportin [43,45]. In 293T cells, Q248H FPN was as susceptible to the actions of hepcidin-25 as wild-type ferroportin [44]. In 293T cells, ferroportin Q248H was less efficient in exporting Fe^{59} than wild-type ferroportin, but the decrease was less pronounced than that caused by the mutant ferroportins encoded by *SLC40A1* A77D, N144H, and V162del [43]. Ferroportin Q248H upregulated cell surface expression of transferrin receptor-1 in the same way as wild-type ferroportin [45]. Expression of ferroportin Q248H in 293T cells led to a similar reduction in total cellular ferritin as did wild-type ferroportin, and to accumulation of similar levels of radiolabeled iron [45]. Taken together, these *in vitro* results are consistent with the present *in vivo* observations that suggest that *SLC40A1* Q248H is associated with a mild clinical phenotype or only causes iron overload in the presence of modifying factors [44,45].

We conclude that the allele frequency of *SLC40A1* Q248H is significantly lower in African Americans than in Native Africans, but is similar in African Americans who reside in central Alabama, the area of Washington, D.C., and in southern California. Although OR estimates of

iron overload in African Americans and Native Africans with Q248H were greater than unity, the increased OR values were not statistically significant. We cannot exclude a causative relationship of iron overload and Q248H in some persons, perhaps by an interaction of Q248H with putative mutations in modifying genes, the ingestion of large quantities of iron, or inflammatory stimuli. Regardless, penetrance of Q248H as a cause of iron overload is probably low.

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Table 1

SLC40A1 Q248H allele frequencies in persons of sub-Saharan African descent

Geographic region (no. of subjects)	Frequency (Q248H alleles/total chromosomes)	[[95% Confidence intervals|]]	[[Reference|]]
Birmingham, Alabama (39) [1]	[[0.0256 (2/78)|]]	[[−0.0104, 0.0617|]]	[4]
Birmingham, Alabama (87) [2]	[[0.0575 (10/174)|]]	[[0.0228, 0.0922|]]	[20]
Washington, D.C. (341) [2]	[[0.0411 (28/682)|]]	[[0.0262, 0.0560|]]	[20]
San Diego, California (571) [3]	[[0.0604 (69/1142)|]]	[[0.0466, 0.0743|]]	[5]
Anonymous U.S. (100) [4]	[[0.0250 (5/200)|]]	[[0.0033, 0.0467|]]	[6]
Anonymous U.S. (100) [5]	[[0.0800 (16/200)|]]	[[0.0423, 0.1177|]]	[5]
[[Zimbabwe (208) |]] [6]	[[0.1010 (42/416)|]]	[[0.0720, 0.1300|]]	[21]
South Africa; Swaziland (51) [7]	[[0.0686 (7/102)|]]	[[0.0187, 0.1186|]]	[6]

[1] Convenience sample of *HFE* C282Y-negative spouses of patients who attended a hematology and oncology clinic.

[2] Participants in a primary-care based hemochromatosis and iron overload screening program who did not have *HFE* C282Y or H63D. In the combined Alabama and Washington, D.C. cohorts, 220 participants were had serum ferritin (SF) >300 ng/mL (men) or >200 ng/mL (women); 208 participants had SF >15 ng/mL, but =300 ng/mL (men) and =200 ng/mL (women).

[3] Persons who attended a health appraisal clinic who were negative for *HFE* C282Y.

[4] Anonymous panel of DNA samples from 100 African Americans.

[5] Anonymous panel of DNA samples from 100 African-Americans obtained from DNA Polymorphism Discovery Resource of the National Human Genome Research Institute of the NIH, Coriell Institute of Medical Research.

[6] Infants and pre-school children attending well-child clinics in Harare, Zimbabwe.

[7] Community participants thought not to have dietary iron overload on the basis of SF <400 ng/mL.

Table 2

SLC40A1 Q248H positivity in persons of sub-Saharan African descent without *HFE* C282Y

Geographic region (no. of subjects)	[[Positive subjects, %|]]	[[Reference|]]
Birmingham, Alabama controls (39) [1]	[[5.1 (2/39)|]]	[4]
Birmingham, Alabama controls (87) [2]	[[11.5 (10/87)|]]	[20]
Washington, D.C. controls (341) [2]	[[7.9 (27/341)|]]	[20]
San Diego, California controls (571) [3]	[[11.7 (67/571)|]]	[5]
African Americans with iron overload (24) [4]	*16.7 (4/24)*	[4–6]
Zimbabwe controls (208) [5]	[[19.2 (40/208)|]]	[21]
South Africa; Swaziland controls (51) [5]	[[13.7 (7/51)|]]	[6]
Native Africans with iron overload (15) [6]	*20.0 (3/15)*	[6]

[1] All were negative for *HFE* C282Y.

[2] Participants in a primary-care based hemochromatosis and iron overload screening program who did not have *HFE* C282Y or H63D. In the combined Alabama and Washington, D.C. cohorts, 220 participants were had serum ferritin (SF) >300 ng/mL (men) or >200 ng/mL (women); 208 participants had SF >15 ng/mL, but =300 ng/mL (men) and =200 ng/mL (women).

[3] These subjects were negative for *HFE* C282Y.

[4] Fifteen patients were from Alabama, five were from California, and four were from the area of Washington, D.C. Three patients were heterozygous and one was homozygous for *SLC40A1* Q248H. All were negative for *HFE* C282Y. One woman, a Q248H heterozygote, was also heterozygous for hemojuvelin A310G (*HFE2*, exon 4, cDNA 929C→G (Ala310Gly)).

[5] Subjects from South Africa and Swaziland were community participants thought not to have dietary iron overload on the basis of SF <400 ng/mL. These subjects were assumed to be negative for *HFE* C282Y based on previous reports of absence of the C282Y allele in sub-Saharan Native Africans [24–27].

[6] These patients resided in South Africa, Zimbabwe, or Swaziland; three were heterozygous for *SLC40A1* Q248H. These subjects were assumed to be negative for *HFE* C282Y based on previous reports of absence of the C282Y allele in sub-Saharan Native Africans [24–27].