Effect of ferroportin Q248H polymorphism on iron status in African children

Ishmael Kasvosve, Zvenyika AR Gomo, Kusum J Nathoo, Petronella Matibe, Boniface Mudenge, Mark Loyevsky, and Victor R Gordeuk

ABSTRACT

Background: Iron deficiency is common in African children, but genetic variations affecting susceptibility have not been identified. The Q248H mutation in ferroportin, a cellular iron exporter regulated by iron status and inflammation, may be associated with high iron stores in African adults.

Objective: The study examined the prevalence of iron deficiency in African children in an area where malaria transmission is low to absent and investigated whether ferroportin Q248H provides protection from iron deficiency.

Design: Complete blood counts, serum markers of iron status and inflammation, and ferroportin Q248H were measured in 208 preschool children in Harare, Zimbabwe. Iron deficiency was defined by serum ferritin and C-reactive protein (CRP) concentrations (definition 1) or by ferritin and the ratio of transferrin receptor to log10 ferritin (definition 2).

Results: Q248H was present in 40 children (38 heterozygotes, 2 homozygotes), elevated CRP was present in 26 (12.5%), and iron deficiency was present in 50 (24.0%) (definition 1) or 55 (26.4%) (definition 2). The interaction between ferroportin Q248H and CRP was significant for ferritin concentrations (P = 0.027) in a 2-factor analysis of variance model. With elevated CRP, the estimated geometric x (SE range) ferritin concentration was 74 (52–106) µg/L for Q248H heterozygotes but 24 (20–30) µg/L for wild-type subjects (P = 0.016). With normal CRP, the ferritin concentration was 16 (14–19) µg/L whether or not the mutation was present. After adjustment for age and weight-for-height z score, the odds ratio (OR) for iron deficiency in Q248H heterozygotes was not significant according to definition 1 (OR: 0.53; 95% CI: 0.18, 1.40; P = 0.222) or definition 2 (OR: 0.39; 95% CI: 0.14, 1.07; P = 0.068).

Conclusions: Any effect of Q248H in protecting against iron deficiency may be observable in children exposed to repeated inflammatory conditions. Further studies of iron status and ferroportin Q248H in African children are needed.


KEY WORDS Ferroportin mutation, African children, iron deficiency, inflammation

INTRODUCTION

Iron deficiency is a common nutritional disorder in children in developing countries (1). Several studies have suggested that iron deficiency in infancy may be associated with impaired cognitive function during the school years (2, 3). In sub-Saharan Africa, low dietary iron and chronic gastrointestinal blood loss due to hookworm infestation are major causes of iron deficiency (4). Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease) may, rarely, cause iron deficiency through gastrointestinal blood loss (5), but no cases have been reported in which genetic disorders caused childhood iron deficiency in the absence of gastrointestinal blood loss.

The SLCO4A1 gene encodes a multiple transmembrane domain protein, ferroportin, which is responsible for the efflux of iron from mature enterocytes of the duodenum and from macrophages of the spleen and bone marrow to plasma (6–9). Macrophages are responsible for recycling iron that is recovered from the catabolism of aged erythrocytes (10). Cellular iron export by ferroportin is regulated by hepcidin, which in turn is regulated by iron stores and inflammation (11, 12).

The cDNA 744G→T substitution in exon 6 of the ferroportin gene, which results in the replacement of glutamine with histidine at position 248 (Q248H), is a common polymorphism in Africans and African Americans that may be associated with a tendency in adults to iron loading (13–15). Cellular studies indicate that, unlike the ferroportin mutations that are associated with macrophage iron overload in white families—eg, A77D (16) and V162del (17)—the Q248H mutation has not been reported to decrease the macrophage membrane expression of ferroportin or to influence the cellular expression of transferrin receptors or the cellular ferritin content in the absence of inflammation (18).

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In the current study, we examined the prevalence of iron deficiency in children in Harare, Zimbabwe, an area where malaria transmission is low to absent (19) and where there is a very low prevalence of hookworm infestation (PK Nathoo, personal communication, 2003). We also investigated whether ferroportin Q248H might provide protection from iron deficiency.

SUBJECTS AND METHODS

Study participants

Two hundred eight infants and preschool children attending well-child clinics in Harare, Zimbabwe, were studied. Body weight and height (length) of the children were measured, and the mother or guardian of each child was asked whether the child had a history of malaria. This was an exploratory study, and the sample size was determined by the limited funds available.

Written informed consent was obtained from the mother or guardian of each child. The institutional review boards of the Medical Research Council of Zimbabwe and of Howard University (Washington, DC) approved the protocol.

Study samples and laboratory measurements

Morning peripheral blood samples (5 mL) were collected into a 2-mL evacuated tube containing K$_3$-EDTA and a 5-mL evacuated tube without the anticoagulant. Complete blood counts were performed by using an automated analyzer (Sysmex, Norderstedt, Germany). The analyzer was calibrated every morning with the use of standards provided by the manufacturer, and the performance of the machine was evaluated by using commercial blood as a quality control. Thin blood smears stained by using Giemsa solution were assessed microscopically for malaria. Serum ferritin, transferrin receptor, and C-reactive protein (CRP) concentrations were measured by using enzyme immunoassays (serum ferritin and transferrin receptors: Ramco Laboratories, Stafford, TX; CRP concentrations: ALPCO Diagnostics, Windham, NH).

Definitions of iron deficiency

Serum ferritin is a sensitive indirect measure of iron stores in healthy persons (20). In the presence of acute and chronic inflammation, the serum ferritin concentration increases independently of iron stores, which reduces its usefulness for a diagnosis of iron deficiency (21). The ratio of transferrin receptor to log$_{10}$ ferritin was shown to identify iron deficiency in the setting of inflammation in a large series that documented iron status by bone marrow examination (22). We employed 2 models to categorize iron status.

Definition 1

In definition 1, iron deficiency was defined as a serum ferritin concentration < 10 µg/L (23). If the serum ferritin concentration was ≥ 10 µg/L, the CRP concentration increased, the subject was considered to have indeterminate iron status. CRP concentrations > 8.2 mg/L were taken to be elevated, as specified by the manufacturer of the kit. The drawback of the use of definition 1 is that, if the child has both elevated CRP and a serum ferritin concentration ≥ 10 µg/L, a determination as to whether the child is iron deficient is not possible.

Definition 2

In definition 2, iron deficiency was defined as a serum ferritin concentration < 10 µg/L or, if the serum ferritin concentration was ≥ 10 µg/L, a transferrin receptor:log$_{10}$ ferritin > 10.8. If the serum ferritin concentration was ≥ 10 µg/L and the transferrin receptor:log$_{10}$ ferritin was 6.7–10.8, then the subject was considered to have indeterminate iron status. (In the current data set, among 46 subjects with serum ferritin concentration < 10 µg/L, the 2.5 percentile value for transferrin receptor:log$_{10}$ ferritin was 6.7. Among 136 participants with serum ferritin concentrations ≥ 10 µg/L and without elevated CRP concentrations, the 97.5 percentile value for transferrin receptor:log$_{10}$ ferritin was 10.8). An advantage of the use of definition 2 is that some children with both elevated CRP and serum ferritin concentrations ≥ 10 µg/L could be classified as to their iron-deficiency status.

Detection of the ferroportin 744G→T mutation

DNA was isolated from leukocytes that were separated from whole blood by using lymphocyte separation medium (MediaTech, Sterling, VA). Exon 6 of ferroportin was amplified by using a set of primers encompassing portions of the introns that flank the exon (forward primer: 5'-CAT CGC CTG TGG CTT TAT TT-3'; reverse primer: 5'-GCT CAC ATC AAG GAG GAG GG-3'). After an initial denaturation at 94 °C for 3 min, a polymerase chain reaction was performed for 5 cycles of heating at 94 °C for 45 s, cooling at 56 °C for 45 s, and heating at 68 °C for 45 s, which were followed by 25 cycles of heating at 94 °C for 45 s, cooling at 52 °C for 45 s, and heating at 68 °C for 45 s and a final cycle of 15 min at 68 °C in a thermocycler (PTC-100; MJ Research Inc, Waltham, MA). The 392-base pair (bp) product was digested with PvuII enzyme (MBI Fermentas, Hanover, MD), and the resulting DNA fragments (252 and 140 bp) were fractionated on 3% agarose gel and detected with ethidium bromide.

Statistical analysis

Weight-for-height z scores were calculated by using EPIINFO software (version 6.04; Centers for Disease Control and Prevention, Atlanta, GA). Statistical analysis was conducted with SYSTAT software (version 11; SYSTAT Software, Inc, Point Richmond, CA). Proportions were compared by using Fisher’s exact test. Continuous variables that followed a skewed distribution were analyzed with the Kruskal-Wallis test or log transformed for analysis of variance (ANOVA) and logistic regression models. The effect of the ferroportin Q248H polymorphism on serum ferritin concentrations was examined by using a 2-factor ANOVA model that included elevated or nonelevated C-reactive protein concentration as the second factor and that adjusted for age and weight-for-height z score. Because there was a significant C-reactive protein × ferroportin Q248H interaction for serum ferritin concentrations (P = 0.027), we also compared serum ferritin concentrations according to ferroportin Q248H status in separate ANOVAs for participants with and without elevated CRP. Each ANOVA model included age and weight-for-height z score as covariates. Logistic regression analysis was used to estimate the association between iron deficiency and Q248H heterozygosity after control for age and weight-for-height z score.
RESULTS

Two hundred eight apparently healthy children aged 3–60 mo who were visiting well-baby clinics in Harare, Zimbabwe, were studied. Eleven children (5.3%) had a history of malaria infection, but none had a positive malaria slide at the time of the study. The clinical and laboratory characteristics of the study population are shown in Table 1. The prevalence of obesity (weight-for-height z score > 2.0; 24) was 3% and that of underweight (weight-for-height z score < −2.0) was 7.2%. The ferroportin Q248H mutation was present in 40 children (38 heterozygotes and 2 homozygotes), absent in 157 individuals and we could not determine the presence of the mutation in 11 subjects due to the poor quality of the DNA recovered. The ferroportin Q248H allele frequency in the population studied was 0.107. Twenty-six (12.5%) children had elevated CRP concentrations. The median CRP concentration was 0.8 mg/L (interquartile range: 0.3, 2.8 mg/L) in Q248H heterozygotes and 0.6 mg/L (interquartile range: 0.1, 2.5 mg/L) in ferroportin wild-type children (P = 0.1).

In a 2-factor ANOVA model, there was a significant ferroportin Q248H × CRP category interaction for serum ferritin concentrations (P = 0.027). After adjustment for age, weight-for-height z score, and that interaction, the estimated P (SE range) serum ferritin concentration was 32 (27–39) μg/L in 38 Q248H heterozygotes and 21 (19–23) μg/L in 157 ferroportin wild-type children (P = 0.033) (Table 2). In children with elevated serum CRP concentrations, the adjusted serum ferritin concentration was 74 (52–106) μg/L in 7 Q248H heterozygotes and 24 (20–30) μg/L in 18 ferroportin wild-type children (P = 0.016), whereas, in children with normal CRP, the estimated geometric mean serum ferritin concentration was 16 μg/L whether the mutation was present or not.

On the basis of definition 1, 50 (24.0%) of the children had iron deficiency and 22 (10.5%) had indeterminate iron status. On the basis of definition 2, 55 (26.4%) of the children had iron deficiency and 21 (10.1%) had indeterminate iron status (Table 1). Under definition 1, 6 of 38 (15.8%) ferroportin heterozygotes had iron deficiency and 42 of 157 (26.8%) ferroportin wild-type subjects had iron deficiency (P = 0.259). Under definition 2, 6 of 38 (15.8%) ferroportin heterozygotes had iron deficiency and 47 of 157 (29.9%) ferroportin wild-type participants had iron deficiency (P = 0.204). In logistic regression modeling, younger age

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic and laboratory features of 208 children attending well-child clinics in Harare, Zimbabwe&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (mo)</strong></td>
<td>24 ± 15&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female [n (%)]</td>
<td>114 (54)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.2 ± 0.7&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight-for-height z score</td>
<td>0.35 (−1.03–0.46)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Obesity&lt;sup&gt;5&lt;/sup&gt; [n (%)]</td>
<td>6 (2.9)</td>
</tr>
<tr>
<td>Underweight&lt;sup&gt;6&lt;/sup&gt; [n (%)]</td>
<td>15 (7.2)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>10.8 ± 1.2&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>RBCs (×10&lt;sup&gt;12&lt;/sup&gt;/μL)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>4.35 ± 0.50&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCV (fL)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>74 ± 7&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum ferritin (μg/L)&lt;sup&gt;9&lt;/sup&gt;</td>
<td>18 (10–31)</td>
</tr>
<tr>
<td>Serum transferrin receptors (mg/L)</td>
<td>6.8 (5.5–8.9)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.7 (0.2–2.6)</td>
</tr>
<tr>
<td>Elevated C-reactive protein&lt;sup&gt;10&lt;/sup&gt; [n (%)]</td>
<td>26 (12.5)</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td></td>
</tr>
<tr>
<td>Definition 1&lt;sup&gt;11&lt;/sup&gt;</td>
<td>50 (24.0)</td>
</tr>
<tr>
<td>Definition 2&lt;sup&gt;12&lt;/sup&gt;</td>
<td>55 (26.4)</td>
</tr>
<tr>
<td>Heterozygotes for ferroportin Q248H [n (%)]</td>
<td>38 (19.3)&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
<tr>
<td>Homozygotes for ferroportin Q248H [n (%)]</td>
<td>2 (1.0)&lt;sup&gt;12&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Two Q248H homozygous subjects were excluded from the analysis.

<sup>2</sup> 2-Factor ANOVA model that includes C-reactive protein as the second factor and adjusts for age and weight-for-height z score. There was a significant interaction between ferroportin Q248H and C-reactive protein category in measuring serum ferritin concentration (P = 0.027).

<sup>3</sup> Geometric x; SE range in parentheses (all such values).

<sup>4</sup> Geometric mean; interquartile range in parentheses (all such values).

<sup>5</sup> Median; interquartile range in parentheses (all such values).

<sup>6</sup> Weight-for-height z score > 2.0.

<sup>7</sup> Weight-for-height z score < −2.0.

<sup>8</sup> For comparison, in the second National Health and Nutrition Examination Survey (NHANES II), for African American children aged 1–2 y, the 5th–95th percentile range for hemoglobin is 10.0–13.0 g/dL and that for MCV is 70–90 fL.

<sup>9</sup> For comparison, in NHANES II, for African American children aged 3–4 y, the 10th–90th percentile range for serum ferritin is 7–39 μg/L.

<sup>10</sup> Concentration >8.2 mg/L.

<sup>11</sup> Definition 1 is serum ferritin < 10 μg/mL. Twenty-two children had serum ferritin ≥ 10 μg/L and elevated C-reactive protein, which was considered to be indeterminate iron status.

<sup>12</sup> Definition 2 is serum ferritin < 10 μg/mL or, if serum ferritin is ≥ 10 μg/mL, a ratio of transferrin receptor to log<sub>10</sub> ferritin > 10.8. Twenty-one children had serum ferritin ≥ 10 μg/L and a ratio of transferrin receptor to log<sub>10</sub> ferritin 6.7–10.8, which was considered to be indeterminate iron status.

<sup>13</sup> n = 198.

<sup>14</sup> n = 197.

<sup>15</sup> 12 n = 157.

<sup>16</sup> 13 n = 38.

| TABLE 2 | Serum ferritin concentration according to ferroportin Q248H<sup>1</sup> |
|----------|---------------------------------
| Wild-type subjects | Q248H heterozygotes | P |
| n | 157 | 38 | 0.033 |
| Serum ferritin (μg/L)<sup>2</sup> | 21 (19–23)<sup>2</sup> | 32 (27–39) | 0.033 |
| Normal C-reactive protein<sup>3</sup> | n | 139 | 31 |
| Serum ferritin (μg/L) | 16 (15–17) | 16 (14–19) | 0.96 |
| Elevated C-reactive protein<sup>4</sup> | n | 18 | 7 |
| Serum ferritin (μg/L) | 24 (20–30) | 74 (52–106) | 0.016 |

<sup>1</sup> Two Q248H homozygous subjects were excluded from the analysis.

<sup>2</sup> 2-Factor ANOVA model that includes C-reactive protein as the second factor and adjusts for age and weight-for-height z score. There was a significant interaction between ferroportin Q248H and C-reactive protein category in measuring serum ferritin concentration (P = 0.027).

<sup>3</sup> Geometric mean; SE range in parentheses (all such values).

<sup>4</sup> Geometric mean; SE range in parentheses (all such values).
was significantly \((P \leq 0.0006)\) associated with iron deficiency under both definitions; a higher weight-for-height \(z\) score was significantly \((P = 0.031)\) associated with iron deficiency only under definition 2 (Table 3). After adjustment for age and weight-for-height \(z\) score, the odds ratio for iron deficiency in ferroportin Q248H heterozygotes compared with that in ferroportin wild-type participants was 0.53 (95% CI: 0.18, 1.40; \(P = 0.222\)) according to definition 1 and 0.39 (95% CI: 0.14, 1.07; \(P = 0.068\)) according to definition 2 (Table 3).

### DISCUSSION

In the current study, we found iron deficiency in \(\approx 25\%\) of children aged \(< 5\) y from an area of Zimbabwe where malaria is not transmitted and hookworm infection is not endemic, but, in an additional \(10\%\) of the children, iron status could not be described with confidence. The prevalence in the study population of subjects with a ferroportin Q248H mutation was 20%, which is similar to our earlier findings in African adults (13, 15). Overall, the statistically adjusted serum ferritin concentration was significantly higher in ferroportin Q248H heterozygotes than in wild-type subjects. This finding was fully accounted for by the markedly higher serum ferritin concentration in Q248H heterozygotes with elevated CRP than in wild-type subjects with elevated CRP. There was a trend to a lower prevalence of iron deficiency in Q248H heterozygotes, but the difference was not statistically significant. In keeping with studies in other countries (25–27), iron deficiency under both definitions was associated with younger age. Iron deficiency under definition 2 was associated with a higher weight-for-height \(z\) score (Table 3), which is consistent with the higher prevalence of iron deficiency among overweight or obese children and adolescents reported by others (25–27).

The ferroportin polymorphism by CRP concentration observed for the serum ferritin concentration is of interest in view of a recent report on the role of hepcidin in regulating ferroportin expression on the membrane of macrophages (12). Hepcidin, which is produced by hepatocytes stimulated by inflammatory cytokines (28), directly interacts with ferroportin on the membrane of macrophages, causing internalization of ferroportin and subsequent degradation of the protein by lysosomes. This process is associated with less export of iron from macrophages; because of this iron retention, ferritin synthesis increases (12). In the current study, the greater elevation in serum ferritin concentration with inflammation (as indicated by increased CRP concentrations) in Q248H heterozygotes than in wild-type subjects is consistent with the possibility that this mutation somehow enhances the interaction of hepcidin with macrophage membrane ferroportin, which results in greater retention of iron. However, the origin of serum ferritin in inflammatory states and the relation of serum ferritin to ferroportin function are not known.

In this study with a small sample size, no firm conclusion regarding a possible protective effect of the ferroportin Q248H variant against iron deficiency can be drawn. The observation of elevated serum ferritin concentrations only in Q248H heterozygotes with elevated CRP raises the possibility that any effect of Q248H in protecting from iron deficiency might be observable mostly in children exposed to repeated or prolonged inflammatory conditions. Given the importance of iron deficiency and the need to understand genetic factors in nutritional disorders, the relation between iron deficiency and ferroportin Q248H should be investigated in a study with a larger sample size and a power analysis that is based on the results of this report.

We thank Lynne McNamara (Witswatersrand University, Johannesburg, South Africa) for her suggestions in developing primers and Anup Madan (Neurogenomics Research Laboratory, University of Iowa) for confirming the ferroportin gene sequence.

IK, ZARG, KJN, and VRG developed the study protocol and the experimental design and obtained funding. IK, KJN, and PM were responsible for subject recruitment and drawing of blood. Serum assays and ferroportin Q248H determinations were carried out by IK and ML, and BM performed statistical analysis of the data. Statistical analysis of the data was performed by VRG and IK. All authors contributed to the preparation of the manuscript. None of the authors had a personal or financial conflict of interest.

### REFERENCES


### TABLE 3

Logistic regression models of the association between iron deficiency and Q248H heterozygosity adjusted for age and weight-for-height \(z\) score

<table>
<thead>
<tr>
<th>Odds ratio for iron deficiency</th>
<th>95% CI</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency: definition 1&lt;sup&gt;1&lt;/sup&gt; 2</td>
<td>0.95</td>
<td>0.92, 0.98</td>
</tr>
<tr>
<td>Increase of 1 mo in age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase of 1.0 in weight-for-height (z) score</td>
<td>1.23</td>
<td>0.93, 1.62</td>
</tr>
<tr>
<td>Presence of ferroportin Q248H heterozygosity</td>
<td>0.53</td>
<td>0.18, 1.40</td>
</tr>
<tr>
<td>Iron deficiency: definition 2&lt;sup&gt;3&lt;/sup&gt; 4</td>
<td>0.53</td>
<td>0.18, 1.40</td>
</tr>
<tr>
<td>Increase of 1 mo in age</td>
<td></td>
<td>0.92, 0.98</td>
</tr>
<tr>
<td>Increase of 1.0 in weight-for-height (z) score</td>
<td>1.37</td>
<td>1.03, 1.83</td>
</tr>
<tr>
<td>Presence of ferroportin Q248H heterozygosity</td>
<td>0.39</td>
<td>0.14, 1.07</td>
</tr>
</tbody>
</table>

<sup>1</sup> Serum ferritin \(< 10 \mu g/L\).
<sup>2</sup> Twenty-two subjects coded as having indeterminate iron status (serum ferritin \(\geq 10 \mu g/L\) and elevated C-reactive protein), and 2 Q248H homozygotes were excluded.
<sup>3</sup> Serum ferritin \(< 10 \mu g/L\) or, if serum ferritin is \(\geq 10 \mu g/L\), a ratio of transferrin receptor to \(\log_{10}\) ferritin \(> 10.8\).
<sup>4</sup> Twenty-one subjects coded as having indeterminate iron status (serum ferritin \(\geq 10 \mu g/L\) and a ratio of transferrin receptor to \(\log_{10}\) ferritin 6.7–10.8), and 2 Q248H homozygotes were excluded.