Effect of ferroportin Q248H polymorphism on iron status in African children1–3

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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 SLC40A1 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. Se-

Inflammation, the serum ferritin concentration increases indepen-

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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 using 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 $\leq 10 \mu$g/L (23). If the serum ferritin concentration was $\geq 10 \mu$g/L and the CRP concentration increased, the subject was considered to have indeterminate iron status. $\log_{10}$ ferritin concentration $\geq 8.2$ 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 $\geq 10 \mu$g/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 $< 10 \mu$g/L or, if the serum ferritin concentration was $\geq 10 \mu$g/L, a transferrin receptor:$\log_{10}$ ferritin $> 10.8$. If the serum ferritin concentration was $\geq 10 \mu$g/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 $< 10 \mu$g/L, the 2.5 percentile value for transferrin receptor:$\log_{10}$ ferritin was 6.7. Among 136 participants with serum ferritin concentrations $\geq 10 \mu$g/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 $\geq 10 \mu$g/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 (Media-Tech, 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 CTC 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 poly-

merase 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 bro-

mide.

Statistical analysis

Weight-for-height $z$ scores were calculated by using EPIC-

INFO 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 X 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.
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) was 23%. Underweight (weight-for-height z score < −2.0) was 2.9%. 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 z (SE range) serum ferritin concentration was 32 (27–39) µg/L in 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 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 22 (10.5%) had indeterminate iron status. 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...
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 relative to that of nonmutant ferroportin Q248H homozygotes with elevated CRP than in wild-type subjects with elevated CRP. The higher serum ferritin concentration in ferroportin Q248H heterozygotes with elevated CRP might be attributable to the protective effect of the ferroportin Q248H mutation against iron deficiency, which is consistent with the possibility that this mutation 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.

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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 complete blood counts. 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


