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Mortality in HIV infection is independently predicted by host iron status and SLC11A1 and HP genotypes, with new evidence of a gene-nutrient interaction\textsuperscript{1–3}

Joann M McDermid, Maarten F Schim van der Loeff, Assan Jaye, Branwen J Hennig, Chris Bates, Jim Todd, Giorgio Sirugo, Adrian V Hill, Hilton C Whittle, and Andrew M Prentice

ABSTRACT

Background: Iron-related genes and iron status may independently contribute to variable HIV outcomes. The nature of the biologically plausible gene-nutrient interaction remains unknown.

Objectives: The objectives were to investigate whether iron-related genotypes and clinically abnormal iron status independently predict mortality in HIV and whether a gene-nutrient interaction exists.

Design: Baseline plasma, DNA, and clinical data were obtained from 1362 HIV-seropositive Gambian adults followed for 11.5 y to ascertain all-cause mortality. Iron status was estimated on the basis of plasma iron, soluble transferrin receptor (sTfR), ferritin, transferrin, transferrin index, and log(sTfR/ferritin). One haptoglobin (HP) and 5 SLC11A1 (NRAMP1) polymorphisms were genotyped.

Results: SLC11A1-SLC3 and CAAA polymorphisms were the best independent genetic predictors of mortality [adjusted mortality rate ratio (95% CI): SLC3:G/C = 0.59 (95% CI: 0.45, 0.85), CAAA:del/ins = 1.51 (95% CI: 1.10, 2.07)]. In an adjusted model that included all polymorphisms, SLC1:199/199, SLC1:other/other, SLC6a:A/A, and CAAA:del/ins were associated with significantly greater mortality, whereas Hp 2–1 and SLC3:G/C were protective. In unadjusted analyses, all biomarker concentrations were significantly associated with mortality. Extending previous findings, both low and elevated iron states were associated with mortality, but the nature of the risk was variable, with linear, inversely linear, and U-shaped associations depending on the biomarker. Mortality was significantly lower in HIV-2 than in HIV-1 infection in the presence of abnormal (low or elevated) iron status. A gene-iron interaction was detected (likelihood-ratio test P = 0.018); however, subject numbers restricted category-specific interpretation.

Conclusions: Iron-related genes, iron status, and their interaction predict mortality in HIV. These findings illustrate the complexity and uncertainty surrounding best practice for managing abnormal iron status and anemia during HIV infection and in regions with a high risk of infection. \textit{Am J Clin Nutr} 2009;90:1–9.

INTRODUCTION

Time-to-disease progression and mortality varies considerably in HIV infection. Fully understanding factors explaining this variation assists in guiding clinical decisions and informing public health policy, while also providing a theoretical basis for the development of novel therapies or justifying existing treatments.

Anemia is associated with the progression and mortality in HIV infection (1–3), but the hemoglobin concentration alone does not reflect the overall or tissue iron exposure. Dramatic iron redistribution occurs during all types of infection as a result of the acute phase response (APR), and iron status can be interpreted as low or elevated depending on which biomarker is chosen (4). Whereas hemoglobin is an important routine clinical marker, it is possible that another biomarker, one indicative of elevated iron status, is a more biologically relevant predictor of HIV outcomes. Indeed, elevated iron status has been associated with increased mortality by our group (5) and others (6) and with increased viral load (7) and potentially increased viral virulence, predisposition to opportunistic infections, and altered immune responses and immunodeficiency (8). Collectively, the evidence that iron metabolism and homeostasis play a role in the outcome of HIV infection is strong, with practical implications in the clinical interpretation and management of iron status and anemia preceding and during infection.

Iron status in HIV infection has thus far been reported in the absence of consideration for potential background genetic effects. Phenotypic analysis of haptoglobin (Hp), encoded by the haptoglobin (HP) gene, has been related to survival time and/or viral loads in HIV infection (7, 9). It has also been associated

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with differential susceptibility, prognosis, or recurrence of the highly prevalent opportunistic infection, tuberculosis (TB) (8, 10). The mechanisms may include functional phenotypic differences in relation to free-hemoglobin binding or iron accumulation that influences the degree of iron-driven oxidative stress or alters the immune response. Because Mycobacterium tuberculosis resides within the phagolysomes of macrophages, any changes in iron status at this level could have significant implications for the host-pathogen iron balance. Solute carrier family 11 member 1 [SLC11A1, or natural resistance-associated macrophage protein-1 (NRAMP1)] is hypothesized to encode a protein acting as a macrophage-level iron gatekeeper. Evidence from diverse populations, albeit predominantly HIV-seronegative subjects, repeatedly shows that SLC11A1 polymorphisms are associated with susceptibility to TB (11). Similarly to reports in the iron-status literature, genetic studies have not considered the effect of host iron status on these observations.

In the present study, mortality in HIV infection was investigated as a function of the independent effects of 6 polymorphisms from 2 iron-related genes. Findings from an earlier report were extended by examining the iron-status and mortality association across the total spectrum of iron states, from low to elevated iron status, and additionally, findings were examined in relation to HIV type. Uniquely, we investigated whether a biologically plausible gene-nutrient interaction was detectable.

SUBJECTS AND METHODS

Study design and population

This study used existing clinical data and archived biological samples that were collected for the Medical Research Council (MRC) Laboratories HIV-seroprevalent Clinical Cohort based in The Gambia, West Africa (12). Subjects recruited between 1 January 1991 and 31 December 2001 were eligible if aged ≥18 y and if peripheral blood mononuclear cells/buffy coats and baseline (defined as ≤90 d after the first HIV-seropositive diagnosis) plasma were available. The MRC/Gambian government ethics committee approved the cohort establishment and biological sample collection, and subsequently, in conjunction with the London School of Hygiene & Tropical Medicine ethics committee, the clinical chemistry and genotyping specific to this study. During the time data were originally collected and archived, viral load testing and antiretroviral therapy were not routinely available in The Gambia.

Mortality ascertainment

The main outcome was all-cause mortality. Follow-up continued at the clinic and/or at home visits throughout the country until 1 June 2002. Subjects were considered lost-to-follow-up if mortality status could not be ascertained during these scheduled visits, and these subjects were censored on the last date on which they were known with certainty to be alive.

Genotyping

HP genotyping was performed by using an allele-specific polymerase chain reaction (PCR)-based method distinguishing the major allelic variants (Hp1 and Hp2) of the z-chain (13).

Iron-status analyses

Details of baseline iron-status estimation in this cohort were described elsewhere (5). In brief, soluble transferrin receptor (sTIR) (R&D Systems, Abingdon, United Kingdom) and ferritin (Immuno-biological Laboratories, Hamburg, Germany) were measured by enzyme-linked immunosorbent assay. Plasma iron was assessed by using an endpoint assay (ABX Diagnostics, Shefford, United Kingdom) and transferrin by turbidimetry (ABX Diagnostics, Shefford, United Kingdom). Composite indexes were calculated as follows: transferrin index = iron (μmol/L)/transferrin (g/L) and TIR:F = log(sTIR/ferritin). Hemoglobin concentrations were measured during routine clinical screening. Iron status was determined at cohort entry, and the effects of subsequent interventions with iron-repletion therapy for anemia were not quantified.

Acute phase response analysis

x1-Antichymotrypsin (ACT) as an indication of the degree of APR was measured by using a nephelometric assay (DakoCytomation Inc, Ely, United Kingdom).

Statistical analyses

Survival analyses were conducted by using Poisson multiplicative regression models with Intercooled Stata 9.2 (StataCorp, College Station, TX). Our null hypothesis was that there were no differences in the probability of mortality across iron-status or genotype categories. This was tested against an alternative hypothesis that there were differences in mortality in categories other than the baseline or reference category. Deciding whether to model a covariate as a continuous or categoric variable often involves a tradeoff between the best-fitting model in statistical terms and a model that is readily interpretable and acceptable by clinicians or patients. In this study, justification of covariate categorization was based on evidence, where available, of clinically relevant cutoffs that are used to define normal or abnormal iron status or commonly used cutoffs in the HIV literature (ie, CD4 cell counts) or developing countries (ie, body mass index (BMI; in kg/m²)). Well-defined cutoffs for each iron-status biomarker by race, sex, age, or under different clinical circumstances are not universally accepted or used between studies. Where possible, we used values that have been reported in the literature from studies that most closely resembled our population. Potential confounders of the iron status (plasma...
Iron, ferritin, transferrin, sTfR, transferrin index, and TIR:F and mortality relation included baseline age (categorized in 4 age groups: 18–25, 26–35, 36–45, and >45 y), sex, BMI (2 categories: ≥18 and <18, based on reference 14), natural log-transformed ACT concentration, absolute CD4 cell count (grouped in 3 categories: >500, 200–500, and <200 cells/µL), HIV type [HIV-1, HIV-2, and both HIV-1 and HIV-2 (HIV-dual infection)], and hemoglobin concentration. These variables, in addition to self-reported ethnicity (5 categories; Mandinka, Jola, Wolof, Fula, and “otherwise”), were included in the adjusted genetic analyses.

Main genetic effects

The gene-mortality relation was analyzed in 3 ways: the genotypes of each polymorphism were analyzed in separate unadjusted models, in separate adjusted-models, and in a single adjusted-model combining polymorphisms from both genes.

Main iron effects

The relation between iron status and mortality was assessed in 3 ways: by analyzing the raw data using biomarker concentrations or index values; by clinically classifying biomarkers according to low, normal, or elevated iron status (15–19) (Table 1); and by stratifying on HIV type.

Joint interaction effect

Based on a priori knowledge, elevated iron status was considered to be associated with greater mortality. According to the cutoffs established from the literature in Table 1, iron status was dichotomized into elevated iron status (≥1) or otherwise (<0). Individual iron risks were then added, giving an overall iron-risk score of low = 0–1, medium = 2–3, and high ≥ 4. A priori knowledge of the probability of mortality associated with each of the genotypes was unavailable. Consequently, an ad hoc classification of the combined genotype risk was created based on the outcome of the independent effect of genotypes on mortality in this study (Table 2, analysis C). High-risk genotypes were coded as Hp:1–1, 2–2; SLC1:199/other, other/other; SLC3:G/G; SLC6a:AA; SLC5b:ins/del; CAAA:del/ins, ins/ins = 1; and otherwise = 0. Scores were added, giving an overall genetic-risk score of low = 0–1; medium = 2–3; high ≥ 4. Regression coefficients and SEs were used to calculate the predicted rate ratios and 95% CIs. The presence of a significant interaction was tested for using the likelihood-ratio test on unadjusted Poisson regression models.

RESULTS

Characteristics of this cohort were described elsewhere (5, 12). In brief, of the 1362 participants, sex was approximately equally distributed (52.7% women), and the mean (±SD) age was 35 ± 10 y. At cohort entry, the median absolute CD4 count for the cohort was 234 [interquartile range (IQR): 83, 469 cells/µL], and HIV-1 (67.3%) was the predominant viral type identified (HIV-2 = 31.3%, HIV-dual = 1.5%). The mean (±SD) hemoglobin concentration (100 ± 23 g/L) and median ACT concentration (0.44; IQR: 0.33, 0.68 g/L) suggest that many were experiencing an APR at the time of blood sampling. The mean (±SD) BMI was significantly lower (P < 0.001) in men (18.5 ± 3.3) than in women (20.2 ± 4.6), as was the median (IQR) absolute CD4 cell count [men: 160 cells/µL (55, 349 cells/µL); women: 317 cells/µL (133, 579 cells/µL); P < 0.001], which may have reflected a tendency toward a longer duration between HIV-seroconversion and HIV diagnosis among men in this cohort.

Mortality

Follow-up continued up to 11.5 y [2714 person-years (PY)], with 713 deaths observed. The proportion of subjects lost to follow-up was 21.6% (median follow-up time: 0.81 y; IQR: 0.15, 2.31 y), with a tendency for these subjects to be female, younger, and healthier at baseline.

Iron-related genes and mortality

We selected 5 SLC11A1 polymorphisms distributed along the entire SLC11A1 genomic sequence and one HP polymorphism for genetic analysis. The genotype distribution is summarized in Table 2. The most frequently observed genotypes in this cohort were Hp = 2–1, SLC1:199/199, SLC3:G/G, SLC6a:G/G, SLC6b: ins/ins, and CAAA:del/ins. The genotypes for all polymorphisms were in Hardy-Weinberg Equilibrium (HWE), with the exception of CAAA (P = 0.025). When examined separately by ethnicity according to mortality outcome, all were in HWE with the exception of the CAAA polymorphism among subjects who were alive at cohort exit (P = 0.012).

The SLC3:G/G genotype was consistently associated with a significantly lower probability of mortality than was the predominant homozygous genotype SLC3:G/G in all genetic models (analyses A-C) (Table 2). After adjustment for multiple confounders (analyses B-C), CAAA:del/ins was also associated with significantly greater mortality than was the CAAA:del/whole genotype. When all polymorphisms of both genes were included in an adjusted model (analysis C), the genetic associations became stronger and the SLC1:199/other, SLC1:other/other, and SLC6a:AA genotypes were also associated with significantly
increased mortality. Conversely, SLC3:G/C and Hp 2–1 were associated with significantly lower mortality.

**Iron status and mortality**

Iron-status biomarkers and associated indexes were important predictors of mortality in HIV infection (Table 3), with all results statistically significant in unadjusted analyses. After adjustment for multiple confounders, iron-status biomarkers alone (transferrin, ferritin, and hemoglobin) or by inclusion of iron and stTfR in the composite indexes transferrin index and TR:F, respectively, were statistically significant predictors of mortality. Because of the nature of iron metabolism, depending on the biomarker of interest, elevated iron status is defined by higher (ie, plasma iron, transferrin index, and ferritin) or lower (ie, stTfR, transferrin, and TR:F) absolute concentrations. The results of this study indicate that increases in the concentration of plasma iron, transferrin index, or ferritin (ie, toward an elevated iron state) were associated with increased mortality [mortality rate ratio (MRR) > 1.0], whereas increases in the concentration of stTfR, transferrin, or TR:F (ie, away from elevated iron state) were protective (MRR < 1.0).

Because single unit changes in the concentration of a particular biomarker are less straightforward to interpret in clinical practice, a second analysis using biomarker concentrations classified according to low, normal, or elevated iron status was conducted (Table 4). In unadjusted models, both low and elevated iron states were significantly associated with increased mortality when compared with normal iron status. After adjustment, both low and elevated iron status were associated with mortality, and elevated iron status estimated by transferrin (adjusted MRR: 1.94; 95% CI: 1.42, 2.66; P < 0.001) and ferritin (adjusted MRR: 1.55; 95% CI: 1.19, 2.02; P = 0.001) were the strongest predictors of increased mortality. As expected, when classified according to increasing severity of anemia, hemoglobin was associated with increased mortality in both unadjusted and adjusted analyses.

Examination of the mortality rates in Table 5 showed an iron status and mortality relation that was of similar direction between the HIV-1 and HIV-2 viruses, and the unadjusted MRR indicated that, without exception, mortality rates were lower with HIV-2 than with HIV-1. After adjustment for many factors that could otherwise account for differences between viral types, the adjusted models provide evidence of differential mortality.
between HIV-2 and HIV-1. However, with the exception of normal iron status estimated by TfR:F, mortality differences between viruses were statistically significant only in the presence of abnormal iron status (ie, lower than or higher than normal iron status).

### Gene-nutrient interaction and mortality

Predicted MRRs are shown in (Table 6), and demonstrate that while increasing joint iron and gene risks are apparent, category-specific conclusions are limited because of the restricted subject numbers with complete genetic and iron-status data. A

#### TABLE 3
Baseline iron status as a predictor of mortality analyzed by concentration

<table>
<thead>
<tr>
<th>Iron-status biomarker</th>
<th>Unadjusted MRR (95% CI) per unit change</th>
<th>P</th>
<th>Adjusted MRR (95% CI) per unit change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTfR (nmol/L)</td>
<td>1.005 (1.002, 1.008)</td>
<td>0.002</td>
<td>0.998 (0.993, 1.004)</td>
<td>0.530</td>
</tr>
<tr>
<td>Iron (μmol/L)</td>
<td>0.942 (0.928, 0.955)</td>
<td>&lt;0.001</td>
<td>1.003 (0.984, 1.023)</td>
<td>0.749</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>0.220 (0.193, 0.250)</td>
<td>&lt;0.001</td>
<td>0.600 (0.474, 0.760)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TfR:F</td>
<td>0.337 (0.301, 0.378)</td>
<td>&lt;0.001</td>
<td>0.701 (0.600, 0.819)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin index (μmol/g)</td>
<td>1.025 (1.022, 1.028)</td>
<td>&lt;0.001</td>
<td>1.033 (1.001, 1.055)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ferritin (μg/L)</td>
<td>1.0015 (1.0014, 1.0016)</td>
<td>&lt;0.001</td>
<td>1.0006 (1.0004, 1.0008)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>0.738 (0.711, 0.765)</td>
<td>&lt;0.001</td>
<td>0.921 (0.878, 0.967)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

1 Mortality rate ratios (MMRs) and 95% CIs were computed by using simple and multivariate Poisson regression analyses. sTfR, soluble transferrin receptor; TfR:F, log(sTfR:ferritin).

2 Adjusted for sex, age, HIV type, absolute CD4 cell counts, BMI, α1-antichymotrypsin, and hemoglobin. Model analysis included the 834–835 subjects with complete cofactor data.

3 Model analysis included the 1015 subjects with complete hemoglobin concentration data.

#### TABLE 4
Baseline iron status as a predictor of mortality analyzed by clinical classification

<table>
<thead>
<tr>
<th>Clinical classification of iron status</th>
<th>Frequency (%)</th>
<th>Unadjusted MRR (95% CI)</th>
<th>P</th>
<th>Adjusted MRR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTfR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>443 (32.5)</td>
<td>1.42 (1.22, 1.67)</td>
<td>&lt;0.001</td>
<td>0.85 (0.67, 1.06)</td>
<td>0.150</td>
</tr>
<tr>
<td>Normal</td>
<td>849 (62.4)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>69 (5.1)</td>
<td>1.19 (0.87, 1.64)</td>
<td>0.280</td>
<td>0.97 (0.62, 1.52)</td>
<td>0.894</td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>698 (51.4)</td>
<td>2.28 (1.96, 2.65)</td>
<td>&lt;0.001</td>
<td>1.05 (0.84, 1.32)</td>
<td>0.676</td>
</tr>
<tr>
<td>Normal</td>
<td>642 (47.3)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>17 (1.3)</td>
<td>3.55 (2.03, 6.18)</td>
<td>&lt;0.001</td>
<td>1.15 (0.36, 3.65)</td>
<td>0.819</td>
</tr>
<tr>
<td>Transferrin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2 (0.1)</td>
<td>—</td>
<td></td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>389 (28.6)</td>
<td>5.32 (4.29, 6.60)</td>
<td>&lt;0.001</td>
<td>1.94 (1.42, 2.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Elevated</td>
<td>968 (71.2)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TRF:F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>139 (10.2)</td>
<td>0.42 (0.30, 0.58)</td>
<td>&lt;0.001</td>
<td>0.61 (0.40, 0.92)</td>
<td>0.020</td>
</tr>
<tr>
<td>Normal</td>
<td>900 (60.1)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>322 (23.7)</td>
<td>4.46 (3.82, 5.21)</td>
<td>&lt;0.001</td>
<td>1.45 (1.13, 1.87)</td>
<td>0.004</td>
</tr>
<tr>
<td>Transferrin index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>564 (41.6)</td>
<td>1.27 (0.98, 1.64)</td>
<td>0.071</td>
<td>1.25 (0.87, 1.78)</td>
<td>0.227</td>
</tr>
<tr>
<td>Normal</td>
<td>160 (11.8)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>633 (46.6)</td>
<td>1.41 (1.10, 1.81)</td>
<td>0.007</td>
<td>1.44 (1.01, 2.04)</td>
<td>0.044</td>
</tr>
<tr>
<td>Ferritin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>129 (9.5)</td>
<td>0.54 (0.37, 0.80)</td>
<td>0.002</td>
<td>0.64 (0.39, 1.03)</td>
<td>0.064</td>
</tr>
<tr>
<td>Normal</td>
<td>574 (42.1)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Elevated</td>
<td>659 (48.4)</td>
<td>4.52 (3.84, 5.32)</td>
<td>&lt;0.001</td>
<td>1.55 (1.19, 2.02)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe anemia</td>
<td>201 (19.8)</td>
<td>4.68 (3.79, 5.77)</td>
<td>&lt;0.001</td>
<td>1.49 (1.16, 1.91)</td>
<td>0.002</td>
</tr>
<tr>
<td>Anemia</td>
<td>288 (28.4)</td>
<td>2.52 (2.06, 3.08)</td>
<td>&lt;0.001</td>
<td>1.27 (1.02, 1.58)</td>
<td>0.036</td>
</tr>
<tr>
<td>Normal</td>
<td>526 (51.8)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

1 Mortality rate ratios (MRRs) and 95% CIs were computed by using simple and multivariate Poisson regression analyses. —, insufficient no. of subjects; sTfR, soluble transferrin receptor; TRF:F, log(sTfR:ferritin).

2 For clinical classification limits, refer to Table 1.

3 Adjusted for sex, age, HIV type, absolute CD4 cell counts, BMI, α1-antichymotrypsin, and hemoglobin. Model analysis included the 834–835 subjects with complete cofactor data.

4 Model analysis included the 1015 subjects with complete hemoglobin concentration data.
comparison of the model without interaction with the model with interaction showed good evidence of an interaction on the multiplicative scale (likelihood-ratio test: \( P = 0.018 \)).

**DISCUSSION**

This is the only reported study that has evaluated polymorphisms of 2 iron-related genes and the gene-nutrient interaction as predictors of mortality in HIV infection to our knowledge. We find that clinically abnormal iron status is an important predictor of mortality, and, for the first time in HIV or indeed any other infection or clinical condition, we observed that \( SLC11A1 \) genotypes also predicted mortality. In this study, we also report the presence of a novel gene-nutrient interaction between the iron-related genetic and iron-status risks in association with mortality.

Similarly to others (1–3), we found an inverse association between hemoglobin and mortality, which highlights the clinical utility of this routinely measured marker. However, we also showed that elevated iron status estimated from several different biomarkers is a strong risk factor for mortality. Together, these findings underscore that iron status at both ends of the continuum exist in HIV infection and that both states are important predictors of mortality.

Unique to this cohort was the ability to evaluate relations between iron and mortality in the 2 different human immunodeficiency viruses. Current evidence indicates a longer survival time with HIV-2 than with HIV-1 infection, but this advantage is limited to persons without advanced immunosuppression (12). The relation between iron status and mortality has not been previously reported in the context of viral type. It is apparent that among subjects with normal iron status, mortality does not significantly differ between HIV-1 and HIV-2. However, among subjects classified as having clinically abnormal iron status, HIV-type becomes an important predictor of mortality. Under these circumstances, HIV-2 has a more favorable mortality outcome than does HIV-1, and this association persists even after the control for degree of immunosuppression and clinical and demographic cofactors.

The APR is associated with low and elevated iron status (4, 22); therefore, it could be argued that biomarkers of iron status are proxies for clinical and subclinical infections. In this study, infection status was assessed and controlled for in the analyses, however, the possibility of residual confounding remains. Even if the biomarkers were proxy indicators, the strong and consistent
association with mortality remains an important clinical observation because extensive laboratory investigations during HIV infection can be impractical in resource-limited settings. Even regular monitoring of the established surrogate markers, CD4 cell counts, and viral load remains economically prohibitive in some resource-limited settings, prompting calls for alternative markers to be developed (23).

The genetic findings we report provide additional support for the need to fully understand iron metabolism and homeostasis in HIV infection. In this study, the heterozygous HP genotype was significantly protective, whereas in a study of European HIV-1 seropositive males, Hp 2–2 phenotypes were associated with shorter survival (9). In healthy males, Hp 2–2 is associated with significantly protective, whereas in a study of European HIV-1 seropositive males, Hp 2–2 phenotypes were associated with higher generalized infectious burdens via mechanisms unrelated to iron status.

For the first time, this study describes the relation between SLC11A1 polymorphisms and mortality. We observed both protective (SLC3G/C) and hazardous genotypes (SLC1:199/other, SLC1:other/other, SLC6a:A/A, and CAAA:del/ins) and demonstrate the relevance of considering these associations in the context of confounding factors. SLC11A1 genotypes may influence mortality through differential susceptibility to infection due to differences in the iron concentration at the level of the macrophage. SLC11A1 and TB susceptibility has been widely studied, and a meta-analysis of HIV-seronegative subjects (11) reported the SLC1:other and SLC6a:A alleles were associated with an increased odds of TB, whereas most of the studies observed that the SLC3:C allele was protective. In the present cohort, subjects with a diagnosis of TB (approximately 18% of the total cohort) had higher mortality than did those without TB (30). Variants in the SLC11A1 gene may influence the function of the SLC11A1 protein, although the exact mechanism remains speculative. SLC11A1 is known to localize to the late endosomal membrane and may affect the early innate immune response to infection through the regulation of iron levels (31, 32). Iron is essential to pathogens, but is also required by the host to generate reactive oxygen/nitrogen intermediates and as a cofactor for superoxide dismutase and catalase that are used to neutralize the cytotoxic effects of the host macrophage oxidative burst (33).

In many instances, gene-environment interactions are thought to be the most important factor in disease onset (34), yet they are rarely investigated. Reports of gene-environment interactions are even rarer in large, longitudinal studies such as the present cohort. Until gene-nutrient interactions are examined and reported, advancing knowledge at the gene-nutrient interface remains restricted. Detecting statistical interaction between iron status and iron-related genes is a novel finding, and, in this study, it appears that mortality is affected by certain genetic backgrounds in the presence of nutritional factors. Limited subject numbers resulted in wide CIs at the category-specific level, and because this analysis was not adjusted for potential confounding variables, interpretations must remain preliminary. Supplementary evidence

### Table 6

<table>
<thead>
<tr>
<th>Genetic risk²</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model without interaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>represented by</td>
<td>MRRₚ × MRRᵣ = MRR (95% CI)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron-risk⁴</td>
<td>Low</td>
<td>Reference group: 1 x 1 = 1</td>
<td>1.13 x 1 = 1.13 (0.80, 1.59)</td>
</tr>
<tr>
<td>Medium</td>
<td>1 x 3.24 = 3.24 (2.47, 4.26)</td>
<td>1.13 x 3.24 = 3.66 (1.99, 6.79)</td>
<td>1.28 x 3.24 = 7.20 (2.25, 7.62)</td>
</tr>
<tr>
<td>High</td>
<td>1 x 7.57 = 7.57 (5.37, 10.68)</td>
<td>1.13 x 7.57 = 8.55 (4.31, 17.02)</td>
<td>1.28 x 7.57 = 9.69 (4.88, 19.09)</td>
</tr>
<tr>
<td><strong>Model with interaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>represented by</td>
<td>MRRₚ × MRRᵣ × MRRᵢ = MRR (95% CI)¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron-risk⁴</td>
<td>Low</td>
<td>Reference group: 1 x 1 = 1</td>
<td>1.61 x 1 = 1.61 (0.83, 3.11)</td>
</tr>
<tr>
<td>Medium</td>
<td>1 x 3.98 x 1 = 3.98 (1.99, 7.96)</td>
<td>1.61 x 3.98 x 0.60 = 3.84 (0.70, 15.53)</td>
<td>1.53 x 3.98 x 1.00 = 6.09 (0.71, 52.76)</td>
</tr>
<tr>
<td>High</td>
<td>1 x 13.78 x 1 = 13.78 (5.95, 31.89)</td>
<td>1.61 x 13.78 x 0.70 = 15.53 (1.27, 188.98)</td>
<td>1.53 x 13.78 x 0.41 = 8.64 (0.72, 103.31)</td>
</tr>
</tbody>
</table>

¹ Predicted MRRs and 95% CIs were calculated by using the observed MRRs and SEs computed by using simple Poisson regression analyses. The likelihood-ratio test was used to assess evidence of interaction on a multiplicative scale, and the calculated P value = 0.018. MRRₚ, predicted MRR of the joint effect of genotypes and iron status; MRRᵣ, predicted MRR of the main effect of genotypes; MRRᵢ, predicted MRR of the main effect of iron status.

² High-risk genotypes were coded as Hp:1–1, HP 2–2; SLC1:199/other, other/other; SLC3:G/G; SLC6a:A/A; SLC6b:ins/del; CAAA:del/ins, ins/ins = 1; otherwise = 0. Individual genotype risks were added, giving an overall genetic-risk score of low = 0–1, medium = 2–3, and high = ≥4.

³ Model analysis included the 586 subjects with complete biomarker and genotype data for all polymorphisms from both genes.

⁴ According to the cutoffs established from the literature in Table 1, elevated iron status was coded as 1 and otherwise as 0. Individual iron risks were added, giving an overall iron-risk score of low = 0–1, medium = 2–3, and high = ≥4.
from additional studies is needed to confirm support for the biologically plausible hypothesis of a gene-nutrient interaction and to more precisely indicate the nature of the effects at the category-specific level. Ultimately, our findings, combined with those of future studies, may contribute to a pooled meta-analysis that is designed to provide such insight.

Limitations associated with this study include the potential for some bias due to the unequal distribution in losses to follow-up. Direct comparisons are difficult because reporting the nature and magnitude of loss-to-follow-up is limited in studies that have been conducted in similar settings. We considered potential discrepancies in genotype calls unlikely to strongly bias findings because all polymorphisms were in HWE, with the exception of CAAA.

Overall, our observations have clinical implications because programmatic as well as less-formalized distribution of iron supplements occurs in resource-limited regions. At this time, however, there is growing concern over the conflicting and insufficient evidence that surrounds the safety or efficacy of iron supplementation during infection or in regions with high infectious burdens (5, 6, 35–40). Some studies have reported that iron supplementation or elevated iron status is not associated with negative outcomes in HIV infection (37, 39, 41), whereas our evidence (5) and that of others (6, 42) suggests the opposite. All-cause mortality is a definitive endpoint, and perhaps the HIV viral load (39, 41) insufficiently assesses the full effect of abnormal iron status if the responsible mechanisms are unrelated to viral load, such as increased susceptibility to opportunistic infections. Another important difference that may partly explain these discrepancies is that we observed effects for both sexes at all stages of infection, whereas other studies were restricted to pregnant women (37) or female hepatitis C—positive injection drug users (39) or included very small numbers of male subjects (41) followed over short time periods.

This study showed that iron-related genes, clinically abnormal iron status, and a gene-nutrient interaction predict mortality among men and women with HIV-1 and HIV-2 infection in The Gambia. Additional research is needed to elucidate the responsible mechanisms. Defining the best practice for the clinical management of iron status and anemia during HIV infection is complex and incomplete.

We thank the study participants and the MRC staff for their contributions to the HIV Clinical Cohort and the staff of the Nutritional Biochemistry Laboratory (MRC Human Nutrition Research) for assistance with the iron-status analyses.

The authors’ responsibilities were as follows—MFSvdL, AJ, HCW, and JMM: performed the data collection, including the acquisition or retrieval of clinical data and biological samples; JMM and CB: performed the genotyping analyses; JMM, MFSvdL, and JT: conducted the data analyses; and JMM: drafted the paper. All authors contributed to the writing of the manuscript and the study conception and design. None of the authors were aware of any potential conflict of interest.

REFERENCES