Common Variants in the TMPRSS6 Gene Alter Hepcidin but not Plasma Iron in Response to Oral Iron in Healthy Gambian Adults: A Recall-by-Genotype Study

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ABSTRACT

Background: The role of genetic determinants in mediating iron status in Africans is not fully understood. Genome-wide association studies in non-African populations have revealed genetic variants in the transmembrane protease serine 6 gene (TMPRSS6) that are associated with the risk of anemia.

Objectives: To investigate the effects of risk alleles for low iron status, namely TMPRSS6 rs2235321, rs855791, and rs4820268, on responses to oral iron in healthy Gambian adults.

Methods: Using a recall-by-genotype design, participants were selected from a pregenotype cohort of 3000 individuals in the Keneba Biobank (Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine). Participants were invited to participate in the study based on 9 genotype combinations obtained from 3 TMPRSS6 single nucleotide polymorphisms (SNPs): rs2235321, rs855791, and rs4820268. The participants fasted overnight and then ingested a single oral dose of ferrous sulfate (130 mg elemental iron). Blood samples were collected prior to iron ingestion and at 2 and 5 h after the oral iron dose. The effects of genotype on hepcidin and plasma iron parameters were assessed.

Results: A total of 251 individuals were enrolled. Homozygous carriers of the major TMPRSS6 alleles at each of the SNPs had higher plasma hepcidin at baseline (rs2235321: GG compared with AA = 9.50 compared with 6.60 ng/mL, P = 0.035; rs855791: GG compared with AG = 9.50 compared with 4.96 ng/mL, P = 0.015; rs4820268: AA compared with GG = 9.50 compared with 3.27 ng/mL, P = 0.002) and at subsequent timepoints. In most subjects, hepcidin concentrations increased following iron ingestion (overall group mean = 4.98 ± 0.98 ng/mL at 5 h, P < 0.001), but double heterozygotes at rs2235321 and rs855791 showed no increase (0.36 ± 0.40 ng/mL at 5 h, P = 0.667).

Conclusions: This study revealed that common TMPRSS6 variants influence hepcidin concentrations, but not iron status indicators either at baseline or following a large oral dose of iron. These results suggest that genetic variations in the TMPRSS6 gene are unlikely to be important contributors to variations in iron status in Africans. This study was registered at clinicaltrials.gov (#NCT03341338).

Keywords: anemia, TMPRSS6, recall-by-genotype, hepcidin, genetic polymorphism, iron absorption

Introduction

Iron supplementation remains the dominant strategy for the prevention and treatment of anemia (1, 2). However, despite decades of implementing this measure together with food-based approaches, the prevalence of anemia remains high, particularly in children and women of reproductive age living in low- and middle-income countries (3).

Matriptase-2 protein encoded by the transmembrane protease serine 6 (TMPRSS6) gene provides tonic suppression of hepcidin, the regulator of iron metabolism (4). Hepcidin blocks the release of intracellular iron by downregulating ferroportin, the only known mammalian...
FIGURE 1 Flowchart illustrating how the study participants were selected into 9 groups based on the 3 candidate TMPRSS6 SNPs. The reference group consisted of individuals who were homozygous for the major alleles of all 3 SNPs. The configuration of the genotype combinations is presented in Supplemental Table 1. Hb, hemoglobin; SNP, single nucleotide polymorphism; TMPRSS6, transmembrane protease serine 6.

cellular iron transporter (5). These effects are especially pronounced in enterocytes and in macrophages, and thus elevated hepcidin is associated with impaired duodenal iron absorption and impaired recycling of aged RBCs (5).

Single nucleotide polymorphisms (SNPs) in TMPRSS6 can lead to decreased function or inactivation of matriptase-2, thus releasing the tonic suppression of hepcidin (HAMP) gene expression (6). This leads to inappropriately elevated hepcidin, which, by blocking iron absorption and recycling, promotes the risk of iron deficiency and anemia (7). Genome-wide association studies have revealed numerous common SNPs in TMPRSS6 that are linked to an increased risk of iron deficiency anemia (IDA) (8).

Three SNPs (rs855791, rs2235321, and rs4820268) have been reported to be associated with low iron status, but mainly in non-African populations (9–12). However, the effects of these SNPs on the response to iron supplementation have not previously been described in Africans. The minor allele frequencies (MAFs) of these SNPs in Africans in the 1000 Genomes project are 10%, 41%, and 28% for rs855791, rs2235321, and rs4820268, respectively (13).

TMPRSS6 rs855791 is a nonsynonymous SNP that alters matriptase 2 protein (14), whereas rs2235321 and rs4820268 are synonymous variants whose direct effects are not clear (14). Although synonymous changes were previously not thought to directly affect phenotype, recent findings show that they can affect protein folding and splicing (15).

In Caucasians, rs855791 is reported to be in high linkage disequilibrium (LD) with rs4820268 and rs2235321 (9, 11), and is in high LD with rs4820268 in Asians (16). However, low LD has been observed between these SNPs in the Africans included in the 1000 Genomes project and in Gambians (17).

In this study, we sought to assess the effects of the 3 common TMPRSS6 SNPs, either individually or combined, on the response to a high dose of oral iron in healthy Gambian adults.

Methods

Study design
The full details of the study design were published in the study protocol (18), and the study was registered at clinicaltrials.gov (NCT03341338). Using a recall-by-genotype approach, participants were enrolled based on their TMPRSS6 rs2235321 (MAF = 43%), rs855791 (MAF = 7%), and rs4820248 (MAF = 27%) genotypes. We selected participants from the Keneba BioBank at Medical Research Council Unit The Gambia (MRCG) at the London School of Hygiene & Tropical Medicine (LSHTM), which contained 3116 pregenotyped participants. Of these 3116 individuals, 1695 met the criteria for inclusion in the present study (Figure 1).
TABLE 1 Configuration of the genotype combinations that formed the bases of participant selection

| Genotype combination | rs2235321 major/minor allele | rs855791 major/minor allele | rs4820268 major/minor allele | Individuals available for selection | Genotype frequency |
|----------------------|------------------------------|-----------------------------|-----------------------------|-------------------------------------|-------------------|
| GG/GG/AA             | G/A                          | G/A                         | A/G                         | 117                                 | 0.066             |
| AA/GG/AA             | G/G                          | G/G                         | A/A                         | 336                                 | 0.190             |
| AG/GG/GA             | A/G                          | G/G                         | G/A                         | 391                                 | 0.229             |
| GG/GG/GA             | G/G                          | G/G                         | G/A                         | 211                                 | 0.129             |
| AG/AG/AA             | A/G                          | A/G                         | A/A                         | 92                                  | 0.054             |
| AG/GG/AA             | A/G                          | G/G                         | A/A                         | 60                                  | 0.044             |
| GG/AG/AA             | G/G                          | A/G                         | A/A                         | 361                                 | 0.211             |
| GG/AG/GA             | G/G                          | G/G                         | G/A                         | 67                                  | 0.035             |
| GG/AG/AA             | G/G                          | G/G                         | A/A                         | 60                                  | 0.033             |

1The genotype combination GG/GG/AA consists of homozygotes for the major alleles at all 3 SNPs, which is the reference group.
2The frequency of each genotype combination in the population with genotype data in the Keneba Biobank (n = 3116).

Genotyping
BioBank participants were previously genotyped using the Infinium 240k Human Exome Beadchip v1.0 and v1.1 (Illumina). Genotype calling was done using data-driven clustering (Genome Studio; Illumina). The TMPRSS6 rs2235321, rs855791, and rs4820268 SNPs were selected based on their previously published associations with measures of iron status.

Genotype combinations
We constructed genotype combinations for each participant from the 3 candidate TMPRSS6 SNPs. This generated a total of 17 genotype combinations (Table 1). Only 9 of these combinations had a sufficient number of individuals to perform grouped analysis. We focused on genotype groups that contained >95 individuals.

Participant selection
Participants were selected based on the combinations obtained from combining the 3 possible genotypes for each of the 3 TMPRSS6 SNPs studied (Figure 1). Individuals were invited to participate if they were between 18 and 50 years of age. Women were excluded if they were breastfeeding or pregnant. Also, individuals who reported to be unwell and those with severe anemia [hemoglobin (Hb) <7 g/dL] were excluded. Individuals who tested positive for malaria were to be excluded but there were none.

Study procedures
Contact details for the potential participants were retrieved from the Kiang West Demographic Database (19). A field worker initially contacted each participant in person or by telephone. Individuals who agreed to participate provided written informed consent (see below) and were invited to the study sites at MRCG Keneba or Fajara, for the investigative procedures.

A baseline blood sample (3 mL: 2.5 mL in lithium heparin and 0.5 mL in EDTA tubes) was taken following an overnight fast. Thereafter, a single dose of 400 mg (2 × 200 mg) ferrous sulfate oral iron, containing 130 mg elemental iron, was given by a study nurse. Participants were observed to ensure that the supplements were taken and the time of ingestion was recorded. Participants were asked to stay at the study site, and blood samples (2.5 mL lithium heparin tubes, at each timepoint) were taken at 2 and 5 h following iron supplementation. In addition, the weight and height of each participant to enable calculation of BMI, and body temperature to assess possible fever, were measured. Plasma iron at the 5-h post iron ingestion sample was used as the primary outcome variable and as a proxy for iron absorption. Differences in hepcidin concentration between genotypes was a secondary outcome.

Laboratory procedures
Full blood count (Medonic M-Series; Boule Medical), malaria rapid test (SD BioLine Malaria Antigen Pf; Standard Diagnostics Inc.), sickling test (sodium metabisulfite method and Hb electrophoresis for confirmation of Hb genotype for positive samples), and glucose-6-phosphate dehydrogenase (G6PD) screening (G6PDHb+; R&D Diagnostics) were performed on the EDTA sample. The lithium heparin samples were centrifuged for 10 min at 277 × g at 4°C, and the plasma stored at −20°C for iron biomarker analysis. Plasma iron, ferritin, unsaturated iron-binding capacity (UIBC), serum soluble transferrin receptor (sTfR), C-reactive protein (CRP), and α-1-glycoprotein were measured using an automated biochemistry analyser (Cobas Integra 400 Plus; Roche Diagnostics). Total iron-binding capacity (TIBC) and transferrin saturation (TSAT) were calculated from UIBC and iron:

\[ TIBC = UIBC + \text{iron and TSAT} = \left(\text{iron/TIBC}\right) \times 100 \] (1)

For all the biochemistry analysis, the analyzer was calibrated using commercial calibrators and controls were analyzed for each parameter.

Hepcidin was quantified using a commercial ELISA (DRG Instruments GmbH) according to the manufacturer’s protocol. To ensure quality of the results, 2 manufacturer-supplied controls (high and low controls) were analyzed alongside the samples in each ELISA plate.

Statistical analysis
Student t test and the Wilcoxon test were used to determine the differences in iron biomarkers between sexes for nonskewed and skewed data, respectively. The χ² test was used to test the differences between categorical data. Linear regression models were used to assess the effects of genetic variants (individual SNPs or genotype combination) on iron markers at each timepoint. The control group (group with individuals homozygous for the major allele at each SNP) was set as the reference in the model. Sex and inflammation status (CRP) were included as
TABLE 2 The number of individuals enrolled into each genotype group

| Genotype group | n   | Number of minor alleles |
|----------------|-----|------------------------|
| Reference group (GG/GG/AA) | 39  | 0                      |
| rs2235321, A/A (AA/GG/AA)    | 35  | 2                      |
| rs2235321, A/G (AG/GG/AA)    | 21  | 1                      |
| rs855791, A/G (GG/AG/AA)     | 28  | 1                      |
| rs4820268, G/A (GG/GG/GA)    | 28  | 1                      |
| rs4820268, G/G (GG/GG/GG)    | 29  | 2                      |
| Double heterozygote (AG/AG/AA: rs2235321 A/G and rs855791 A/G) | 13  | 2                      |
| Double heterozygote (AG/GG/GA: rs2235321 A/G and rs4820268 G/A) | 38  | 2                      |
| Double heterozygote (GG/AG/GA: rs855791 A/G and rs4820268 G/A) | 20  | 2                      |
| Total number of study participants | 251 |                         |

1Details of the genotype group configuration are presented in Table 1.

covariates to account for their known influence on iron status (20, 21). Also, G6PD and sickle-cell anemia were accounted for. Furthermore, account for the influence of baseline iron on the response to the oral iron dose, baseline ferritin, was included as a covariate in the analysis. Skewed data were log-transformed. Bonferroni correction was applied to adjust for multiple testing. Statistical analyses were conducted using the R statistical software (22).

Ethics statement

This study was approved by the MRCG Scientific Coordinating Committee and the MRCG at LSHTM/Gambia Government Joint Ethics Committee (SCC1429), and the LSHTM Ethics Committee (11,679). A fieldworker administered a copy of the study information sheet to each participant. Individuals who could not read had it translated to a language they understood in the presence of an independent witness. Each participant provided written informed consent prior to enrolling into the study, and those who could not write provided a thumbprint. To ensure confidentiality of the participants, all the samples and forms were anonymized by allocating study numbers.

Results

A total of 251 individuals were enrolled in the study, and the number of individuals enrolled in each genotype group is shown in Table 2. Due

TABLE 3 Baseline characteristics of the study population

| Variable | All (n = 251) | F (n = 191) | M (n = 60) | P value (F vs. M) |
|----------|--------------|-------------|-----------|------------------|
| Age, y   | 29.0 (18.0–50.0) | 33.0 (18.0–50.0) | 22.0 (18.0–40.0) | <0.001          |
| Plasma iron, μmol/L | 13.5 (0.4–57.1) | 12.3 (0.4–57.1) | 15.6 (4.4–36.2) | 0.012           |
| Hepcidin, ng/mL | 2.89 (0.05–71.7) | 2.46 (0.05–34.60) | 3.86 (0.09–71.71) | 0.009           |
| TSAT, %   | 21.6 (6.0–100.0) | 20.8 (6.0–100) | 26.0 (6.0–57.8) | 0.001           |
| Transferrin, g/L | 2.82 ± 0.58 | 2.91 ± 0.55 | 2.55 ± 0.61 | <0.001          |
| UIBC, μmol/L | 46.9 (21.1–105.1) | 48.2 (21.1–105.1) | 41.7 (22.2–86.2) | <0.001          |
| TIBC, μmol/L | 61.4 (10.3–112.2) | 62.2 (10.3–112.2) | 57.0 (35.9–94.7) | 0.002           |
| Ferritin, μg/L | 31.0 (0.0–237.7) | 25.5 (0.0–237.7) | 50.0 (7.8–160.4) | <0.001          |
| sTfR, mg/L | 4.00 (1.90–11.32) | 4.11 (1.90–11.32) | 3.54 (2.01–7.62) | 0.011           |
| CRP, mg/L | 0.80 (0.03–26.95) | 0.91 (0.03–26.95) | 0.63 (0.05–13.40) | 0.175           |
| Hb, g/dL  | 12.3 ± 1.5 | 12.0 ± 1.3 | 13.3 ± 1.6 | <0.001          |
| RBC number (×10^12) | 4.4 ± 0.6 | 4.3 ± 0.5 | 4.8 ± 0.6 | <0.001          |
| MCV, fL   | 81.1 ± 6.1 | 81.1 ± 6.0 | 80.0 ± 6.2 | 0.882           |
| Hematocrit, % | 35.9 ± 4.5 | 35.0 ± 3.8 | 38.8 ± 5.0 | <0.001          |
| RDW, %    | 12.2 ± 1.2 | 12.2 ± 1.2 | 12.3 ± 1.0 | 0.615           |
| MCH, pg   | 27.9 ± 2.5 | 28.0 ± 2.5 | 27.8 ± 2.5 | 0.675           |
| MCHC, g/dL | 34.4 ± 1.2 | 34.5 ± 1.2 | 34.3 ± 1.1 | 0.424           |
| BMI, kg/m² | 21.3 (14.4–39.1) | 22.0 (14.4–39.1) | 19.5 (16.3–25.8) | <0.001          |
| Sickle-cell trait (AS/AA) | 21/251 | 17/191 | 4/60 | 0.742           |
| G6PD deficiency (carrier/noncarrier) | 4/177 | 0/133 | 4/44 | 0.003           |

1Student t test was used to determine the differences between parametric data, Wilcoxon test was used for nonparametric data, and \( \chi^2 \) was used to test the differences between categorical data. AA, sickle homozygotes wildtype; AS, sickle heterozygotes, CRP, C-reactive protein; F, females; G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; M, males; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; sTfR, serum soluble transferrin receptor; TIBC, total iron binding capacity; TSAT, transferrin saturation; UIBC, unsaturated iron binding capacity.

2Skewed data listed as medians (ranges).
3Normally distributed data presented as means ± SD.
4Categorical data presented as proportions.
5Individuals not tested for G6PD deficiency (n = 74).
Effects of TMPRSS6 variants on plasma iron

**FIGURE 2** The differences in plasma iron concentrations between genotype of individual SNPs (A) and between the reference group and the double heterozygotes (B), before and after iron ingestion. Plasma iron significantly increased in all the genotype groups after the iron dose. The reference is the genotype group with individuals that carry 2 major alleles for all 3 SNPs. The configuration of the genotype groups is presented in Table 1. The horizontal lines showing the P values indicate the difference between the lowest and the highest concentrations. The y-axis represents means of plasma iron, and the error bars represent SE of the means.

mostly to outward migration of males we had more females (76%) than males. The WHO Hb cutoffs (<12.0 g/dL and <13.0 g/dL, for nonpregnant women and men, respectively) were applied to determine anemia in the study population. Eighty (31.9%) of the participants were anemic, and 54 (64%) of these were females. From the 80 anemic individuals, 30 (37.5%) were iron deficient (had IDA) (ferritin <15 μg/L and CRP <5.0 g/L), with an overall 11.9% of the study population iron deficient. Twenty-six (86.7%) of the iron-deficient individuals were female. The study was conducted in the Kiang West District of the Gambia, which is a rural area ~200 km from the capital city (Banjul). Due to the low economic activity in this area, it is common for men to leave for the urban areas in search of employment. The baseline characteristics of the study participants are presented in Table 3.

The effects of genotype on plasma iron concentrations before and after iron ingestion

Plasma iron increased significantly in all the genotype groups after the iron dose (Figure 2). No significant differences were observed between the genotypes of individual SNPs (Figure 3A) and between double heterozygotes and the reference group, in plasma iron both before and after the iron dose (Figure 3B).
FIGURE 3 The differences between genotypes of individual SNPs (rs2235321, rs85579, and rs4820268) (A), and between the double heterozygotes and the reference group (B), in plasma iron concentration before and after iron ingestion. The reference group consisted of individuals who are homozygotes for the major allele for all 3 SNPs (rs2235321 GG, rs855791 GG, and rs4820268 AA) (see Tables 1 and 2). There were no significant differences between the genotype groups in iron concentration either before or after the iron dose. The y-axis represents means of plasma iron, and the error bars represent SE of the means. SNP, single nucleotide polymorphism.

The effects of genotype on hepcidin concentrations before and after iron ingestion

There were significant differences between genotypes of individual SNPs on hepcidin (Figure 4A). For each of the SNPs, carriers of the homozygous major alleles (rs2235321 GG, rs855791 GG, and rs4820268 AA; Figure 4A) had higher hepcidin concentrations than individuals with the minor alleles, both before and after the iron dose. In addition, when comparing double heterozygotes and the reference group, the latter had the highest hepcidin concentrations at all the timepoints, and this significantly differed from the genotype group AG/AG/AA (Figure 4B). The genotype group AG/AG/AA had the lowest hepcidin concentration both before and after the iron dose (Figure 4B).

There was an increase in hepcidin concentration in all the genotype groups, following the iron dose (Figure 5A), except in 1 group (AG/AG/AA; double heterozygotes at rs2235321 and rs855791) (Figure 5B). The individuals in the genotype group AG/AG/AA had the lowest hepcidin concentrations at baseline, and this remained unchanged 5 h after iron ingestion (Figure 5B). Also, carriers of the genotype rs2235321 AA increased their hepcidin concentrations after the iron dose, but the difference between the baseline and 5 h was not statistically significant ($P = 0.060$) (Figure 5A).

The effects of genotype on TSAT, TIBC, UIBC, ferritin, sTfR, transferrin, and hematology traits

There were significant differences between genotypes of each of the SNPs in TIBC and UIBC at baseline, but these differences were not detected at 2 and 5 h after the iron dose (Supplemental Table 1). There were no significant differences between the genotypes for any of the
FIGURE 4  Hepcidin concentrations before and after the iron dose, within the genotypes of individual SNPs (A) and in the double heterozygotes and the reference group (B). Hepcidin increased in all the genotype groups except in the genotype group AG/AG/AA (double heterozygotes at rs2235321 and rs855791). The horizontal lines showing the P values indicate the difference between the lowest and highest concentrations. The y-axis represents means of the hepcidin concentrations, and the error bars represent SE of the means. SNP, single nucleotide polymorphism.

SNPs in TSAT, transferrin, ferritin, or sTfR either before or after iron ingestion (Supplemental Table 1). Furthermore, there were no differences between genotypes for any of the SNPs on any of the hematological traits (Supplemental Table 2). There were no significant differences between the double heterozygotes and the reference group in TSAT, TIBC, and UIBC.

Discussion

We used a candidate genotype approach to recall individuals with variant alleles of 3 TMPRSS6 SNPs previously associated with iron imbalances. We hypothesized that carriers of risk alleles previously reported to be associated with low iron status would have inappropriately elevated hepcidin concentrations and thus impair oral iron absorption.

Our study participants were healthy individuals, and we used the increase in plasma iron at 5 h after ingestion of the iron dose as a proxy to measure response to oral iron. We found that all subjects increased their plasma iron concentrations and TSAT levels at 5h, but there were no differences between genotypes individually or in combination. Therefore, we could not establish that carriage of low-iron risk alleles from any one of these SNPs impairs the postdose response to oral iron.

The TMPRSS6 rs855791 A allele has been widely associated with iron-refractory iron deficiency anemia in Caucasians and Asians (23–26). Similarly, the rs2235321 A and rs4820268 G alleles have been linked to the risk of iron deficiency, including in African populations.
FIGURE 5 Differences in hepcidin concentrations between genotypes of individual SNPs (A), and between double heterozygotes and the reference group (B), before and after iron ingestion. For each SNP, carriage of the homozygous major alleles is associated with elevated hepcidin concentrations. The genotype group AG/AG/AA had the lowest hepcidin concentration both before and after the iron dose (B). The reference group consists of individuals without any minor allele from the 3 SNPs (rs2235321 GG, rs855791 GG, and rs4820268 AA) (see Tables 1 and 2). The group AG/AG/AA (consisting of heterozygotes at rs2235321 and rs855791, and homozygous major allele at rs4820268) did not change their hepcidin concentration after the iron dose. The horizontal lines showing the \( P \) values in (A) indicate the differences between the highest and lowest concentrations for each SNP. The y-axis represents the means of the hepcidin concentrations, and the error bars represent SE of the means. SNP, single nucleotide polymorphism.

In a meta-analysis, Gichohi-Wainaina and colleagues (8) reported the rs855791 A allele to be associated with decreased Hb and ferritin concentrations across all the populations they studied. Therefore, we expected these SNPs to have an effect on plasma iron biomarkers either at baseline or on the response to the iron dose. However, as with the plasma iron, we did not find any effects of these SNPs on ferritin, TSAT, or any other iron biomarker either before or after iron ingestion. Also, there were no differences in hematological traits in our study.

In most subjects plasma hepcidin showed the anticipated acute rise in response to the administered iron dose. For rs855791, we observed an unexpected result where, at baseline, GG carriers had higher hepcidin concentrations compared with AG. The same trend was observed at 5 h post iron ingestion. This contradicts what has been reported about this SNP in other populations. The rs855791 AA (homozygous for the minor allele) has been associated with elevated hepcidin concentrations accompanied by decreased TSAT and serum iron in Europeans (9). In the study conducted by Nai and colleagues (9), hepcidin decreased in a dose-dependent manner, with rs855791 AA having higher hepcidin concentrations than AG and GG carriers. Our results contradict this finding, because we observed carriers of rs855791 GG to have higher hepcidin concentrations than AG. However, due to its low MAF, we were unable to include rs855791 AA in the study. Most of the previous reports on rs855791 were obtained from studies of non-African
populations. Hence, our results suggest that rs855791 can have a different effect on hepcidin concentrations against the different genetic background of West Africans.

There have been a number of recent studies on the effects of TMPRSS6 variants on iron status in different populations. A study of Pakistani women of reproductive age found that rs855791 T allele (A on the reverse strand) is associated with the risk of IDA (29). However, in the present study, we did not have individuals with the T allele. In a study of South African chronic kidney disease patients, Nalado and colleagues (30) found that the rs855791 alleles had no effect on IDA. This finding is similar to our results on this SNP. Also, in an iron absorption study in Taiwanese women using stable iron isotopes, Buerkli et al. (31) reported that the TMPRSS6 rs855791 alters iron absorption, and that the carriers of the C alleles absorbed iron better than T allele carriers. Our study was short (5 h) and we did not use stable isotopes.

From the analysis of genotype combinations, we did not find any group that differed significantly from the reference (GG/GG/AA: rs2235321 GG, rs855791 GG, and rs4820268 AA) in plasma iron concentrations or TSAT levels. However, we observed that carriers of the genotype combination AG/AG/AA (simultaneous carriage of rs2235321 AG, rs855791 AG, and rs4820268 AA) maintained a low mean hepcidin concentration for ≤5 h, despite exposure to a high iron dose. The plasma iron concentrations of this group rose significantly by 5 h post iron ingestion, whereas the hepcidin concentrations remained constant. This contradicts the clear rises in hepcidin concentrations shown for most subjects in this study, and widely reported in the literature (32, 33). An acute rise in hepcidin concentrations in response to oral iron is the normal feedback mechanism to halt further iron absorption when optimal concentrations are reached (34). In IDA, low concentrations of hepcidin promote iron absorption, but this is still accompanied by acute elevation of hepcidin (35). Further studies of why this group lacks the acute hepcidin response, so clearly evident in the other subjects, are warranted and might provide insights into the response of hepcidin.

A strength of this study is that, using a recall-by-genotype strategy, we concentrated on informative individuals by focusing on known carriers of our genotypes of interest. This is an efficient method to identify genotype-phenotype relations with improved statistical power and to eliminate the effects of confounders (35). A major limitation of our study was the low MAF of the variant most widely described in the literature (rs855791), and this prevented us from studying homozygotes for the minor allele. Other limitations include the fact that the subjects were relatively iron replete with high hepcidin concentrations. We therefore cannot exclude the possibility that the gene variants studied might have differential effects under conditions of iron deficiency. Likewise it is possible that responses to a lower (more physiological) dose of iron might differ from the results reported here. Using the postprandial change in plasma iron as the primary outcome has strengths and weaknesses. It permitted large numbers of measurements to be made and is a relevant measure of the acute response to iron administration, but is only a proxy measure of intestinal iron absorption (36). As in any study of this type the ability to detect genotype effects will be blunted by natural variance caused by differences in diet, nutritional status of other micronutrients potentially affecting iron status, and potential effects of inflammation. Epistatic influences of other genes cannot be excluded.

From this study, we conclude that common TMPRSS6 variants influence hepcidin, but not postprandial iron status (a proxy for oral iron absorption). It therefore seems unlikely that genetic variations in the TMPRSS6 gene are important contributors to differences in iron status in this, and likely other, African populations.

Acknowledgments
We thank Alhassan Colley, MRCG at LSHTM Keneba Laboratory, for conducting the biochemistry analysis; and Bakary Sonko and Lamin Y Darboe, MRCG at LSHTM, for managing the study database. We also thank the population of West Kiang District, The Gambia, for participating in the study. The authors acknowledge Dr Branwen Henning for setting up the Keneba Biobank.

The authors’ responsibilities were as follows—MWJ, AMP, and CC: conceptualized the study; MWJ, SC, and CC: designed the study; MWJ and AS: conducted data collection and laboratory analyses; MWJ, AMP, and CC: performed the data analysis; MWJ: wrote the manuscript; and all authors: read and approved the final manuscript.

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