Brief report

A genome-wide association analysis of serum iron concentrations

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To investigate genetic variants that affect iron concentrations in persons not affected by overt genetic disorders of iron metabolism, a genome-wide association study was conducted in the InCHIANTI Study (N = 1206) and the Baltimore Longitudinal Study of Aging (N = 713). The top 2 single-nucleotide polymorphisms were examined for replication in the Women’s Health and Aging Study (WHAS) I and II (N = 569). The single-nucleotide polymorphism most strongly associated with lower serum iron concentration was rs4820268 (P = 5.12 × 10−9), located in exon 13 of the transmembrane protease serine 6 (TMPRSS6) gene, an enzyme that promotes iron absorption and recycling by inhibiting hepcidin antimicrobial peptide transcription. The allele associated with lower iron concentrations was also associated with lower hemoglobin levels, smaller red cells, and more variability in red cell size (high red blood cell distribution width). Our results confirm the association of TMPRSS6 variants with iron level and provide further evidence of association with other anemia-related phenotypes. (Blood. 2010;115:94-96)

Introduction

Iron is an important cofactor for enzymes performing basic functions in human physiology.1 Iron deficiency has important pathologic consequences including but not limited to anemia.2 Iron is also toxic and can react with oxygen species to form chemically active free radicals that damage macromolecules and cellular organelles.3 To avoid both deficiencies and toxicity, iron homeostasis is tightly regulated.

Iron balance is maintained through regulation of dietary iron uptake and systemic distribution with only very small quantities eliminated through bleeding and shedding of the intestinal mucosa.4 Studies have suggested that variability in iron concentrations is in part genetically determined with heritability estimates of 20% to 30%.5,6

Over the past decade, heritable, overt pathologic iron deficiencies and iron overload have been attributed to mutations in a number of key genes that control iron homeostasis.1 However, whether iron levels are affected by genetic variants in subjects who are not affected by these Mendelian diseases is unclear. To address this question, we conducted a genome-wide association study (GWAS) in the InCHIANTI and the Baltimore Longitudinal Study of Aging (BLSA) and confirmed our results in the Women’s Health and Aging Study (WHAS).

Methods

Study subjects

The InCHIANTI study is a population-based epidemiologic study performed in a sample of the population living in the Chianti region of Tuscany, Italy.7 The BLSA study is a population-based study conducted in a sample of volunteers predominantly from the Baltimore–Washington, DC, area.7 The WHAS I and II are companion prospective, observational studies of the causes and consequences of disability in older women.5,10 The 3 studies were approved by the institutional review boards at their respective institutions.

Iron-related measurements

Serum iron was measured using a colorimetric assay (Roche Diagnostics; InCHIANTI;WHAS) or Fe slide method (VITROS 750; Johnson & Johnson; BLSA). Serum ferritin was measured using Quest Diagnostics Laboratory (formerly Ciba-Corning Laboratories; WHAS), chemiluminescent immunoassay (Abbott Diagnostic; INCHIANTI) or an immunoassay-type 2-stage sandwich method using 2 antiferritin antibodies (Advia Centaur, Bayer; BLSA). Other traits (hemoglobin, hematocrit, red blood cell width, mean corpuscular volume, red blood cell count, and platelets) were assessed using autoanalyzer SYMSMEX SE-9000 (Sysmex Corporation; InCHIANTI), coulter hematology analyzer (WHAS), and SYMSMEX XE-series (Sysmex Corporation).

Genotyping

Genome-wide genotyping of the InCHIANTI and BLSA was assessed using the Illumina Infinium HumanHap 550K.11,12 Association analysis was conducted on 475 322 single-nucleotide polymorphisms (SNPs) that passed quality control (minor allele frequency ≥ 1%, genotyping completeness ≥ 99%, and Hardy Weinberg-equilibrium > 0.0001). Genotyping of rs855791 and rs4820268 in WHAS was performed using Applied Biosystems TaqMan Assays on Demand.


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A meta-analysis of the 2 GWAS and the 2 replication studies resulted in a genome-wide significant P value of 4.16 × 10⁻⁸ (rs855791) and 5.12 × 10⁻⁹ (rs4820268; Table 2). An additional 14 SNPs within 10 kb of TMPRSS6 were represented in the GWAS panel (supplemental Table 2). The SNPs in moderate LD with the top SNPs (r² = 0.2-0.3 in Hapmap CEU population) were modestly associated with serum iron concentrations (rs2235320, rs5756504).

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### Results and discussion

The mean serum iron concentrations were comparable across the study populations (Table 1). Because the population examined in this study represents older persons, the genetic effects on iron resulting from menstrual blood loss were minimized. Polymorphisms associated with serum iron levels with P value less than 1 × 10⁻⁵ are listed in supplemental Table 1 (available on the Blood website; see the Supplemental Materials link at the top of the online article). A common polymorphism on chromosome 22 in the transmembrane serine protease 6 (TMPRSS6) or matriptase-2 gene reached genome-wide significance (supplemental Table 1; supplemental Figure 1). Variant rs855791 in exon 17 showed the strongest association (P = 3.93 × 10⁻⁵), confirming results from a recent GWAS study of iron levels. The second strongest SNP, rs4820268 on exon 13, was in linkage disequilibrium (LD) with rs855791 (r² = 0.9), representing the same signal. Both rs855791 (P = .037) and rs4820268 (P = .003) were significantly associated with iron concentrations in combined analysis of WHAS I and II studies. A meta-analysis of the 2 GWAS and the 2 replication studies resulted in a genome-wide significant P value of 4.16 × 10⁻⁸ (rs855791) and 5.12 × 10⁻⁹ (rs4820268; Table 2). An additional 14 SNPs within 10 kb of TMPRSS6 were represented in the GWAS panel (supplemental Table 2). The SNPs in moderate LD with the top SNPs (r² = 0.2-0.3 in Hapmap CEU population) were modestly associated with serum iron concentrations (rs2235320, rs5756504).

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concentrations. Of the candidate SNPs was significantly associated with iron absorption and use were associated with iron concentrations in this study (supplemental Table 3). After adjusting for multiple comparisons, none of the candidate SNPs was significantly associated with iron concentrations.

TMPRSS6 regulates iron absorption through suppression of hepcidin antimicrobial peptide (HAMP). Hepcidin is a key regulator of iron homeostasis that induces degradation of ferroportin (SLC40A1), the only transporter known to facilitate elemental iron egress from macrophages and enterocytes. During iron deficiency, hepcidin is downregulated to promote ferroportin-mediated iron uptake and correct the deficiency. Several genes are required for appropriate regulation of the LDL-receptor class A-like (LDLRa) domain (rs4820268) and a nonsense SNP within the trypsin-like serine protease domain (rs855791). The synonymous SNP most probably is in LD with a functional SNP within or near TMPRSS6. Functional analysis of common variants within TMPRSS6, in particular rs855791, is warranted.

We examined whether the TMPRSS6 SNPs were associated with other iron-related hematologic values in the 4 studies (Table 2). Although the TMPRSS6 SNPs were not associated with anemia prevalence (assessed using hemoglobin values), the alleles associated with lower iron concentrations were also associated with lower mean corpuscular volume, lower hemoglobin levels, and higher red blood cell distribution width. Whether this genetic background is associated with higher risk of developing iron-deficiency anemia should be tested in future studies.

In conclusion, we confirm a previously reported TMPRSS6 locus in association with lower serum iron concentrations. These variants were also significantly associated with smaller red cells, lower hemoglobin levels, and higher red blood cell distribution width. Because this gene is directly involved in the regulation of dietary iron absorption and use, this SNP may be an informative marker to identify a subgroup at increased risk of iron-restricted erythropoiesis as a consequence of inefficient absorption of iron from dietary sources.

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Authorship


Conflict-of-interest disclosure: The authors declare no competing financial interests.

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